Photodynamic Killing of Human Cancer Cells with Smart Photosensitizer Materials and An Endoscopic Implement for Singlet Oxygen Delivery

Mihaela N. Minnis

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PHOTODYNAMIC KILLING OF HUMAN CANCER CELLS WITH SMART PHOTOSENSITIZER MATERIALS AND AN ENDOSCOPIC IMPLEMENT FOR SINGLET OXYGEN DELIVERY

by

MIHAELA NICOLETA MINNIS

A DISSERTATION SUBMITTED TO THE GRADUATE FACULTY IN CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, THE CITY UNIVERSITY OF NEW YORK

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Mihaela Minnis

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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THE CITY UNIVERSITY OF NEW YORK
Abstract

PHOTODYNAMIC KILLING OF HUMAN CANCER CELLS WITH SMART PHOTOSENSITIZER MATERIALS AND AN ENDOSCOPIC IMPLEMENT FOR SINGLET OXYGEN DELIVERY

by

MIHAELA MINNIS

Adviser: Alexander Greer

Abstract: The thesis describes progress on probe tips for a microoptic device for the precise delivery of the components necessary for photodynamic therapy (PDT) in a highly localized and controllable fashion. The thesis also summarizes results of a photosensitized oxidation study. The work focused on i) developing a photoactive fluoropolymer surface that will release sensitizer drug molecule for use in PDT, ii) designing new probe tips surfaces for use as sensitizer support for a microoptic PDT device, iii) exploring strategies for covalent attachment of sensitizer and model compounds to Teflon/PVA surfaces with the aim of being coupled with our microoptic device, and iv) initiating photosensitized dissociation of peroxides at silica surface by triplet sensitizer energy transfer as a strategy to break peroxide O–O bonds for RO• release to study the oxidative process.

Development of a photoactive fluoropolymer surface that releases sensitizer drug molecule was achieved. The surface is a Teflon/poly(vinyl alcohol) (PVA) nanocomposite bearing a photoreleasable PEGylated photosensitizer that generates \(^{1}O_2\) (\(^{1}\Delta_g\)) [chlorin e\(_6\) methoxy tri(ethylene glycol) trimester]. We observed that the Teflon-like fluorinated surface showed resistance to drug adsorption and that there is also an increase in ground state oxygen by the material. The relative surface efficiency to photorelease the PEGylated sensitizer was slightly higher for the nanocomposite when compared with fluorinated PVG surface. We also found that the presence of C–F bonds in the polymers was beneficial for high \(^{1}\)O\(_2\) solubility, repelling action, and low physical quenching of \(^{1}\)O\(_2\). The fluoropolymer could be shaped into device tips to discharge controlled sensitizer and \(^{1}\)O\(_2\) quantities for tissue repair or pointsource photodynamic therapy in vivo.
Two different types of probe tips material for a micooptic PDT device were successfully synthesized and their mechanical strength, stability and efficiency for their used as sensitizer support was determined. The surfaces were Teflon-like nanocomposite, made of polyvinyl alcohol (PVA) and polytetrafluoroethylene (PTFE) and a silica monolith, prepared using an acid based catalyzed sol-gel method. Two types of sensitizersilica monolith surfaces were synthesized: sensitizersilica monolith and sensitizer doped silica monolith. Both xerogel surfaces, in presence of light and oxygen, successfully generated singlet oxygen in water which was detected by chemical trapping with alkene, trans-2-methyl-2-pentenoic acid, and anthracene.

PDT sensitizers and model compounds were covalently attached to the plain or succinic acid functionalized Teflon/PVA surface. We were successful in (1) synthesizing 9-bromomethylanthracene-Teflon/PVA heterogeneous surfaces via an alkylation reaction (2) covalently attaching chlorin e₆ onto the plain Teflon/PVA surface via bromopropanol linker (3) synthesizing a hybrid Teflon/PVA-9-anthracenemethanol surface by covalently attaching 9-anthracenemethanol via an esterification reaction to the Teflon/PVA surface, previously modified with succinic acid and (4) synthesizing Teflon/PVA probe tip with photo detachable pheophorbide molecules. The heterogeneous surface when placed in solution, in presence of light and oxygen, was observed to show coloration of butanol solution where 99% sensitizer detached from the probe tip.

Lastly, we explored photochemical reactions at silica surface with the focus on photosensitized dissociation of peroxides. Triplet sensitizer energy transfer as a strategy to break peroxide O–O bonds for RO∙ release was confirmed. We successfully observed the photosensitized dissociation of dicumyl peroxide, upon irradiation of 4,4’-dimethyl benzyl sensitizer adsorbed on fume silica particles. The highest amount of cleaved peroxide (25.3%) was detected in solution when particles were loaded with dicumyl peroxide: 4,4’-dimethyl benzyl in 1:10 ratio.
ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my mentor, Professor Alexander Greer. His constant encouragement and valuable guidance were powerful motivations in the development of this thesis. He is an amazing adviser and one of the kindest, most understanding people I know. I have been profoundly inspired by Professor Greer’s ground breaking projects and, in the process of working with him, I have significantly expended my knowledge and professionalism. I believe in his work and wish him the great success he deserves.

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without him. He changed my life. I thank him for encouraging me to believe in myself and making me be a better person. I value his advice, his kind soul and his love.

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TABLE OF CONTENTS

Contents

Chapter 1:  

Introduction and Background  

1.1 Precise Tumor Removal: Advances and Limitations  

1.2 Need for a Pointsource Device for Precise Tumor Removal  

1.3 Photodynamic Therapy (PDT)  

1.4 Microoptic Device in Photodynamic Therapy  

1.5 Overview of Probe Tips Used for the Microoptic Device  

1.6 References  

Chapter 2:  

Teflon-like Photoactive Surfaces that Release Sensitizer Drug Molecules Based on $^1$O₂ Reactions  

2.1 Introduction  

2.2 Results and Discussion  

2.2.1 Fabrication and Post-Modification of Fluorinated Surfaces for Efficient Drug Photorelease
Chapter 2: Sensitizer Photorelease from the Fluorinated Solid Supports

2.2.2 PEGylated Sensitizer Photorelease from the Fluorinated Solid Supports 13

2.2.3 Testing the Fluorinated Surfaces for Resistance to Sensitizer Drug Adsorption 18

2.2.4 Dioxygen Solubility Enhancements Caused by the Fluorinated Surfaces 21

2.2.5 Mechanism of Sensitizer Photorelease from the Teflon-like Surfaces 23

2.2.6 Additional Studies on the PEGylated Sensitizer-Teflon/PVA Surface 25

2.3 Conclusion 33

2.4 References 34

Chapter 3: Design of New Probe Tips for the Microoptic Device: Teflon-like and Silica Xerogel Probe Tips 39

3.1 Introduction 39

3.2 Results and Discussion 40

3.2.1 Synthesis of Teflon-like Probe Tips for the Microoptic Device 40

3.2.1.1 Characterization of Teflon-like Probes Surface 41

3.2.1.2 Stability of Teflon/PVA Surface 43

3.2.1.3 Oxygen Permeability through the New Teflon-like Tip 46

3.2.1.4 Evaluation of Self-Repelling Properties of the New Teflon Based Microtip 50

3.2.2 Preparation of Porous Silica Probe Tips via Sol-Gel 53

3.2.2.1 Synthesis of Silica Monoliths via Sol-Gel Acid-Based Catalyzed Method 54

3.2.2.2 Increasing Silica Monoliths Strength: Drying Control Chemical Agents 55
3.2.2.3 Synthesis of Dye Doped Glass and Singlet Oxygen Generation

3.2.2.4 Preparation of Sol-Gel Hybrid Probe Tips with Adsorbed Cationic Porphyrine and Detection of Singlet Oxygen at the Silica Surface

3.3 Conclusion

3.4 References

Chapter 4: Functionalization of Teflon/PVA Microptic Tip Surface Using Model Compounds and Chlorophyll Derivatives

4.1 Introduction

4.2 Results and Discussion

4.2.1 Alkylation of Teflon/PVA Surface Using 9-Bromomethyl Anthracene

4.2.2 Immobilization of chlorin e\textsubscript{6} to the Teflon/PVA Surface via Non-Photocleavable Linker

4.2.3 Covalent Attachment of Pheophorbide Ester to the Teflon/PVA Surface

4.2.4 Teflon/PVA Surface Modification: Functionalization of Teflon/PVA with Succinic Acid.

4.2.4.1 Covalent Attachment and Loading of 9-antracenemethanol to the Succinic Acid Functionalized Teflon/PVA Microtip

4.3 Conclusion

4.4 References

Chapter 5: Photooxidation Reactions at Fumed Silica Surface

5.1 Introduction

5.2 Results and Discussion

5.2.1 Synthesis of Sensitizer and Peroxide Silica Adsorbed Particles
5.2.2 Photosensitized Dissociation of Peroxide  

5.2.3 Peroxide Quantification after Particle Desorption in Acetonitrile  

5.3 Conclusion  

5.4 References
### LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>brine</td>
<td>Saturated aqueous sodium chloride solution</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>CCl₄</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deuterated chloroform</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethyl amino pyridine</td>
</tr>
<tr>
<td>D₂O</td>
<td>Deuterium oxide</td>
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<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>dd</td>
<td>Double of doublet</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift in ppm</td>
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<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>¹H</td>
<td>NMR Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>LiAlH₄</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
</tbody>
</table>
MeOH  Methanol
mg    Milligram
min   Minute
mL    Milliliter
mmol  Millimole
NaH   Sodium hydride
ppm   Parts per million
PTFE  Polytetrafluoroethylene
PVA   Polyvinyl alcohol
PVG   Porous vycor glass
q     Quartet
rt    Room temperature
s     Singlet
SA    Succinic acid
t     Triplet
THF   Tetrahydrofuran
TLC   Thin layer chromatography
UV-Vis Ultraviolet and visible spectroscopy
# LIST OF FIGURES

## Chapter 1

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2</td>
</tr>
<tr>
<td>Schematic diagram of PDT process involving Type I and Type II photochemistry, with Type II having the major contribution. Abbreviations: ISC intersystem crossing.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>4</td>
</tr>
<tr>
<td>Concept of the pointsource handheld PDT device for precise tumor removal: (1) illuminator-fiber coupling, (2) compressed oxygen-to-fiber coupling via a flare T-valve to a borosilicate fiber optic consisting of a Teflon gas flow tube, (3) porous Vycor glass (PVG) cap-to-fiber coupling, (4) photocleavable sensitizer solid, (5) internally flowing light and oxygen, externally produced $^1$O$_2$, [2+2] cycloaddition at the alkene site, (6) cleavage of sensitizer 3 free from the probe tip via the scission of dioxetane 2, and (7) production of cofragment 4 and hydrolysis byproduct.</td>
<td></td>
</tr>
</tbody>
</table>

## Chapter 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>9</td>
</tr>
<tr>
<td>Design of the surfaces tailored for $^1$O$_2$-initiated sensitizer drug photorelease reactions. The sensitizers, chlorin derivatives that were PEGylated with triethylene glycol, were bound to surface OH groups of (A) fluorinated silica (Vycor glass monolith coated with nonafluorosilane), and (B) a Teflon/polyvinyl alcohol nanocomposite. Red light irradiation leads to the attack of $^1$O$_2$ with the ethene resulting in the formation of a dioxetane. A second step follows with cleavage to release the sensitizer, although the sensitizer can adhere to the surface in an adsorbed state depending on the surface properties.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>11</td>
</tr>
<tr>
<td>Synthesis of spacer triPEG chlorin 5.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>12</td>
</tr>
<tr>
<td>Synthesis of sensitizer-conjugated organically to Teflon/PVA nanocomposite 10 and by-product formation of surface diester sites.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>13</td>
</tr>
<tr>
<td>Synthesis of sensitizer-conjugated organically to fluorinated silica</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>14</td>
</tr>
<tr>
<td>Photooxidation of fluorinated silica 7 and Teflon/PVA nanocomposite 10 leads to the photorelease of sensitizer 13.</td>
<td></td>
</tr>
</tbody>
</table>
8. Evolution of the sensitizer 13 which photo-departed away from the fluorinated silica 7 (●) and Teflon/PVA 9 (■) into n-butanol solution at 25 °C. Error bars represent the standard deviation obtained from 3 measurements.

9. Effect of surface type on the adsorption of sensitizer. Adsorption profile of sensitizer (initial concentration of chlorin e6-triPEG ester 10 µM). % Adsorption of dye (chlorin e6-triPEG ester) to the fluorinated Vycor (lighter grey bar), succinic acid functionalized Teflon/PVA (darker grey bar) and Teflon/PVA (black bar) surfaces were studied for 90 min. 27 % dye adsorption for fluorinated Vycor was found, whereas only 1.25 % adsorption occurred for Teflon/PVA polymer after 90 min.

10. Percent of PEGylated sensitizer adsorbed onto fluorinated surfaces [fluorinated silica 7 (▲), Teflon/PVA 8 (■), and succinic acid functionalized Teflon-PVA 9 (●)] in n-butanol. The samples were immersed in n-butanol, which contained 10 µM sensitizer and then samples were removed at the indicated times.

11. Mechanism of photorelease of sensitizer drug bound to (A) fluorinated silica 7 and (B) Teflon/PVA 10. The green spheres are fluorine atoms.

12. UV absorption spectra of reaction mixture containing chlorin-triPEG before (black) and after reaction (red).

13. SEM image at 1720X (left) and 20000X (right) magnification of plain Teflon/PVA before sensitizer covalent attachment showing a smooth PVA surface with high content of submicron PTFE particles.

14. SEM image at 1720X (left) and 20000X (right) magnification of chlorin e6-Teflon/PVA surface showing changes in surface morphology upon sensitizer attachment: presence of aggregates on the polymer surface (left image) and an increase in surface area and groves formation (right image).

15. Time course of photorelease of chlorin e6-triPEG in 1-butanol solution arising from photooxidative cleavage and departure from the fiber optic device tip. The absorption spectra show the Q band at 660 nm. Dye loading was 40 nmoles per 0.250 g cap.

16. Percent of chlorin-triPEG photorelease from the Teflon/PVA surface in 1-butanol with internal irradiation.

17. Chemical structure of chlorin e6-trimethylester.
18. Adsorption of chlorin e6-trimethylester at t=0 s (top) and t=3 h (bottom) onto native Vycor (left), fluorinated Vycor (middle) and Teflon/PVA (right) after 3 h.

Chapter 3

Figure Page No

19. PVA and PTFE components included in the new surface material. 41

20. Chemical structure of the nonionic surfactant, TERGITOL™ TMN-10 present in the PTFE emulsion. 41

21. IR spectra of polymer having different Teflon contents have been shown Teflon/PVA 6:1 (green), Teflon/PVA 4:1 (blue) and Teflon/PVA 2:1 (red). IR spectral peak for –OH stretching frequency at 3296.77 cm\(^{-1}\) decreases as the amount of PVA contents decreases in the polymer. Also an increase of peak intensity for the C-F stretching vibrations of PTFE is observed as the amount of PTFE is higher in the polymer. 42

22. Stability of Teflon polymers in 100 % DMSO at room temperature after 24 h: (left) Teflon/PVA 6:1, (middle) Teflon/PVA 4:1 and (right) Teflon/PVA 2:1. 44

23. Stability of Teflon/PVA in 100 % DMSO with time 24h (left vial), 6 h (middle vial), and initial time (right). 44

24. Polymer Teflon/PVA 2:1 (left), Teflon/PVA 4:1 (middle), Teflon/PVA 6:1 (right) after toluene reflux at 110 0°C, showing a stable Teflon/PVA 6:1 surface and slight degraded Teflon/PVA surfaces with 4:1 and 2:1 ratios. 45

25. Teflon/ PVA (6:1) surface after oven drying at 180 0°C, showing PVA decomposition. 45

26. Time course of pO\(_2\) pressure in 1mL of water when oxygen is delivered through Teflon/PVA (black) or PVG (red) cap into the aqueous solution at 7 psi. 49

27. Percent increase of oxygen pressure with time when O\(_2\) is delivered through the Teflon/PVA or PVG cap in 1 mL 1of water. Teflon/PVA (black) and PVG (red) cap. 50
Image showing repellent properties of Teflon/PVA (6:1 first row, 4:1 middle row, 2:1 bottom row) surfaces to various dyes that were sitting in a 2.0 mL solution containing (10 mM dye solutions: rose bengal, phthalocyanine tetralsulfonic acid, meso tetra porphine tetratosylate, rhodine G7, TPP (mesotetra phenyl porphine), pheophorbide $a$ and chlorin $e_6$ after 3h.

Structure of Meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate.

Photooxidation of anthracene dipropionate dianion at the sol-gel surface.

Time course of anthracene disappearance in solution during photolysis, monitored by UV at 378 nm.

Percent anthracene dipropionate dianion disappearance with time.

Photooxidation of trans-2-methyl-2-pentenoate anion.

Chapter 4

<table>
<thead>
<tr>
<th>Figure</th>
<th>Bioconjugation of chlorophyll derivatives to Teflon/PVA surface via nucleophilic substitution.</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure</th>
<th>Alkylation of PVA hydrogel using alkyl halides.</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure</th>
<th>Chemistry of attachment of 9-hydroxymethyl anthracene to the Teflon/PVA surface.</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure</th>
<th>Covalent attachment of 9-bromomethylantracene to the Teflon-PVA polymer has been shown where fluorine and hydroxyl groups are randomly distributed on the polymer surface.</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure</th>
<th>Synthesis of 9-bromomethylantracene from 9-anthracenemethanol in presence of PBr3.</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure</th>
<th>Teflon/PVA surface before (white) and after (yellow) 9-bromomethylanthracene covalent attachment.</th>
</tr>
</thead>
</table>
40. Proposed scheme for covalent attachment of chlorin e₆ to the Teflon/PVA surface via bromopropanol linker.

41. HPLC chromatogram of the starting material, chlorin e₆, tₑ=12.6 min.

42. HPLC chromatogram of the final reaction mixture after covalent attachment of bromopropanol linker to chlorin e₆ showing the formation of mono and diester.

43. Mass spectra of chlorin e₆ monoester.

44. Teflon/PVA surface after chlorin e₆ covalent attachment.

45. Synthetic approach for Teflon/PVA surface functionalization with PPa sensitizer.

46. Teflon/PVA surface before (white) and after (dark brown) sensitizer attachment.

47. Time profile for the photocleavage of 133 nmol sensitizer from the Teflon/PVA surface into 1.0 mL of 1-octanol.

48. Functionalization of Teflon/PVA with succinic acid by esterification reaction.

49. The FTIR spectrum of plain Teflon/PVA in which the 3100-3600 cm⁻¹ band is assigned to O-H stretching (characteristic to PVA) while the bands near 1203 and 1147 cm⁻¹ were assigned to the C-F stretching vibrations of PTFE.

50. Infrared spectrum of Teflon/PVA esterified with succinic acid showing the ester absorption at 1717 cm⁻¹.

51. Infrared spectrum of succinic acid.

52. Attachment of 9-antracenemethanol to the functionalized Teflon/PVA surface.

53. Succinic acid functionalized Teflon/PVA surface, before (white) and after (yellow) 9-antracenemethanol covalent attachment.

54. UV spectrum of DMSO solution containing the dissolved antracene functionalized Teflon/PVA surface, absorbance maxima 366 nm.

55. Calibration plot of 9-antracenemethanol in DMSO.
Chapter 5

Figure | Page No
--- | ---
56. Intermediates generated in the photosensitized oxidation process. | 87
57. Image of the particles modified to release singlet oxygen and alkoxy radicals upon visible light irradiation. The particles are in contact with solution (biphasic) or in a superhydrophobic surface (triphasic). Singlet oxygen and alkoxy radicals are produced in the vicinity of the sensitizer, which are fully wetted or partially wetted or dry before departing into the solution. | 88
58. CG-MS spectra of desorbed peroxide and sensitizer SiO$_2$ particles before photoirradiation in acetonitrile. | 91
59. CG-MS spectra of desorbed peroxide and sensitizer SiO$_2$ particles after photoirradiation in acetonitrile-d sowing new peak appearance at ~ 16 min, confirming the photosensitized dissociation of peroxides. | 92

LIST OF TABLES

Chapter 2

Table | Page No
--- | ---
1. Yield of Photorelease by Teflon/PVA and Fluorinated Silica Photooxidation. | 14
2. Photorelease of Sensitizer on Fluorinated Surfaces. | 16
3. Absolute number of bonds or functional groups on the fabricated surfaces per gram$^a$ | 17
4. Adsorption of triPEG in $n$-butanol. | 20
5. Rates of Reaction with Singlet Oxygen by Native Silica, Fluorinated Silica 6, Teflon/PVA 8, and Teflon in Acetone. | 21

7. Percent of sensitizer photorelease from the Teflon/PVA surface in butanol. The 0.250 cap was loaded with 40 nmoles. 28

8. Amount of sensitizer loaded and photorelease in 75 min from Teflon/PVA surface. 29

9. Nanomoles and percent adsorption of chlorin e_6-trimethylester onto native Vycor, Fluorinated Vycor and Teflon/PVA surfaces in dichloromethane. 30

10. Nanomoles and percent adsorption of chlorin e_6-trimethylester onto native Vycor, fluorinated Vycor and Teflon/PVA surfaces in 1-butanol. 32

**Chapter 3**

**Table** | **Page No**
--- | ---
11. Characteristics of PTFE emulsion. | 41
12. Polymers stability in organic solvents at room temperature for 24 h. | 43
13. Oxygen permeability through porous PVG fiber tip. | 46
14. Quantities of oxygen delivered through Teflon-PVA cap attached to fiber optic cable. | 47
15. Changes in oxygen pressure and pO_2 levels when O_2 is delivered through the Teflon/PVA or PVG cap in 1 mL of water. | 48
16. Nanomoles and percent adsorption of dyes adsorbed onto Teflon/PVA (6:1, 4:1, 2:1) surfaces in different solvents after 3h. Loadings onto the Teflon/PVA ranged from 0.8 nmol TPP onto Teflon/PVA 6:1 to 139 nmoles of meso tetra porphine tetratosylate adsorbed onto Teflon/PVA 2:1. | 51
17. Percent adsorption of rhodin G7 onto native PVG, fluorinated PVG and Teflon/PVA surface. The Teflon/PVA samples were immersed in water (10mL) which contained rhodin G7 (10^{-5} M). The samples were removed from the solution after 4 h. | 52
18. Mole of meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate doped in 0.1g xerogel. 57

19. Absorbance of 5x10^{-5} M anthracene solution at 30 seconds intervals. 59

20. Mole of meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate loaded on approximate 0.1g xerogel. 61

21. Percent yield of alkene oxidation after 4 h. 62

Chapter 4

Table 22. Moles of 9-bromomethylanthracene attached to the Teflon-like surface. 69

Table 23. Nanomoles and percent sensitizer release from the Teflon/PVA surface in 1.0 mL butanol after 2 h. Amount of sensitizer present in solution was detected by UV. 78

Table 24. Loading of succinic acid onto the Teflon/PVA surface. 83

Chapter 5

Table 25. Amounts of dicumyl peroxide and 4,4’-dimethyl benzyl adsorbed on fumed silica particles. 89

Table 26. Percent conversion calculated based on peroxide peak disappearance (average of 3 experiments). Biphenyl was used as internal standard. 93
I dedicate this thesis to my husband, Jamie, my son, Gavril, and my mom and dad for their constant support and unconditional love. I love you all so much.
Chapter 1: Introduction and Background

1.1 Precise Tumor Removal: Advances and Limitations. There are clinical examples that require high-precision technology to eradicate tumors located next to vital tissue. Sites that are often inaccessible or risky for treatment by traditional surgical and medical methods include major blood vessels such as the critical areas of the brain, portions of the eye and carotid arteries.

1.2 Need for a Pointsource Device for Precise Tumor Removal. Minimizing damage to critical normal nearby tissue during tumor removal makes precision a very important aspect in treatment of cancers.\textsuperscript{1,2} A high need of use of a pointsource device (vs conventional systemic delivery of photosensitizer) would be in treatment of brain tumors where sensitizer delivery is problematic due to blood-brain barrier. An improvement in PDT would be the invention of a device with good precision in cell killing and delivering of singlet oxygen. A pointsource hand-held PDT device (Figure 2), was invented by our research group which photoreleases sensitizer and generates singlet oxygen to targeted areas with high precision. “Pointsource” refers to the level of precision in which sensitizer and singlet oxygen are delivered to the specific tumor sites. In summary, there is a critical need for treatment strategies that would generate singlet oxygen site-specifically with high precision in abundant concentrations for enhanced tumor destruction.

1.3 Photodynamic Therapy (PDT). Photodynamic therapy has developed as a powerful method for the treatment of cancer, such as skin and esophageal cancers. Photodynamic therapy is a photochemistry-based therapeutic approach, which uses a photosensitizer (light-activated chemical) and light of appropriate wavelength to impart cytotoxicity via the generation of singlet oxygen (reactive molecular species). In PDT, a photosensitizer (porphyrin, chlorine, etc.) absorbs light of a specific wavelength and is excited to its singlet state. Then it undergoes internal conversion to the relatively long lived and low energetic triplet state that transfers its energy to the molecular oxygen. Molecular oxygen thereby is excited to its singlet state. (See Figure 1)

Sensitizer excitation is usually attained via a one photon transition between the ground state, and a singlet excited state. Relaxation of the singlet excited state produces the lowest excited singlet state of the sensitizer while intersystem crossing generates the sensitizer triplet state. The lifetime of the sensitizer triplet state is longer (ms) than that of the sensitizer singlet state (ns) allowing this excited state to react in one of two ways, known as types I and II mechanisms.
Schematic diagram of PDT process involving type I and type II photochemistry is shown in Figure 1. Type II mechanism has the major contribution in cancer cell killing where singlet oxygen is generated via an energy transfer process during a collision of the excited sensitizer with triplet oxygen. There is also a minor contribution in cancer cell killing from Type I which involves hydrogen-atom abstraction or electron-transfer between the excited sensitizer and a substrate, yielding free radicals which can react with oxygen to form an active oxygen species such as the superoxide radical anion.

Figure 1. Schematic diagram of PDT process involving Type I and Type II photochemistry, with Type II having the major contribution. Abbreviations: ISC intersystem crossing.

Singlet oxygen, $^1$O$_2$, is a highly reactive species, very short lived and has a very short diffusion distance. So a very important factor in getting efficient cytotoxic effect is the site of its generation. Lifetime and diffusion distance of singlet oxygen are largely dependent on the environment. For example the lifetime of singlet oxygen in water is 3.5 µs, in n-octanol it is 19 µs$^3$ while in lipid media is in the range of 13-35 µs.$^4$ The diffusion distance is highly dependent on the lifetime of singlet oxygen, and data shows that singlet oxygen tends to diffuse more in an oleaginous environment. In PDT singlet oxygen is the main component which is cytotoxic and reacts with biomolecules, such as olefinic sites of lipids, amino acids and DNA base pairs$^5$ causing cancer cell death.

The photoactivated photosensitizer (PS) excites oxygen to the (a$^1$Δ) state via a type-II photochemical pathway.$^6$ PDT is gaining popularity around the world as either a primary or an
adjunctive treatment for solid cancers of the head and neck, brain, lung, pancreas, intra-peritoneal cavity, breast, prostate and skin, as reviewed by Hopper. Previous studies provide strong evidence that singlet oxygen is the active species in cancer cell necrosis. Present photodynamic therapy (PDT) methods use systemic administration of dyes followed by site-specific illumination using a light delivery system. Typically, the range of wavelengths for the therapeutic activation of the photosensitizer is 600–800 nm, to avoid interference by endogenous chromophores and achieve maximum tissue penetration. Conventionally, photodynamic therapy uses intravenously injected photosensitizers to generate singlet oxygen ($^1$O$_2$) for the treatment of tumors.

The fundamental challenges associated with photodynamic therapy are (i) the free photosensitizer must be efficiently eliminated from the body, (ii) sufficient sensitizer and light intensity must be achieved for the sensitization reaction to generate an adequate concentration of singlet oxygen at the target site, and (iii) target sites are often inaccessible because the diseased tissue is next to vital tissue (iv) some tumors are hypoxic and PDT fails in treating them.

1.4 Microoptic Device in Photodynamic Therapy. Difficulties exist in surgically removing tumors from complex sites, such as when tumors are directly adjacent to vital organs. A fiber optic-based device for targeted delivery of cytotoxic singlet oxygen for the potential application in diseases, such as brain tumors where exquisite precision is needed in the treatment. This project is pioneering in that, to date, all photodynamic therapy strategies have involved the systemic administration of a sensitizer; our work would therefore be the first to cleave a photosensitizer from the end of a fiber optic device through which O$_2$ flows. The microoptic device developed (hybrid photosensitizer/fiber optic) precisely delivers cytotoxic singlet oxygen in vivo. Its improved singlet oxygen delivery and selectivity will ultimately be of great benefit for removing tumors directly adjacent to vital tissue.

1.5 Overview of Probe Tips Used for the Microoptic Device. At first we experimented with a PVG sensitizer-immobilized fiber optic device as a unique way to deactivate bacteria and photooxidize compounds in water. A new technology, developed in 2011, featured a fiber optic device capable of site-specific sensitizer release. The new microoptic device consists of a maneuverable PVG mini-probe tip that sparges O$_2$ gas and photodetaches pheophorbide-a (sensitizer) molecules. Singlet oxygen is produced at the probe tip surface which reacts with an
alkene spacer group releasing sensitizer upon fragmentation of a dioxetane intermediate as can be seen from Figure 2. Fiber optic drug delivery is a unique system that guides the photosensitizer to release in a specific location.

Figure 2. Concept of the pointsource handheld PDT device for precise tumor removal: (1) illuminator-to-fiber coupling, (2) compressed oxygen-to-fiber coupling via a flare T-valve to a borosilicate fiber optic consisting of a Teflon gas flow tube, (3) porous Vycor glass (PVG) cap-to-fiber coupling, (4) photocleavable sensitizer solid, (5) internally flowing light and oxygen, externally produced $^{1}\text{O}_2$, [2+2] cycloaddition at the alkene site, (6) cleavage of sensitizer 3 free from the probe tip via the scission of dioxetane 2, and (7) production of co-fragment 4 and hydrolysis byproduct.
Our first generation singlet oxygen fiber optic delivery device consisted of a porous Vycor cap coated with \textit{meso}-tetra(\textit{N}-methyl-4-pyridyl)porphine as sensitizer placed at the end of a hollow core photonic bandgap fiber. The hollow fiber flowed O\textsubscript{2} gas and a guided 532 nm light from a continuous-wave or pulse laser. Singlet oxygen was successfully delivered at the distal end of the fiber into aqueous solution with a concentration of 20 fM\textsuperscript{16}.

In order to increase the efficiency and diffusion distance of the \textsuperscript{1}O\textsubscript{2} into the surroundings, a second generation fiber optic delivery device system was developed that sparges O\textsubscript{2} and photodetaches a sensitizer. This new device internally flows triplet oxygen and externally produces singlet oxygen, causing a reaction at the (Z)-1,2-dialkoxyethene spacer group, freeing a pheophorbide sensitizer upon the fragmentation of a reactive dioxetane intermediate\textsuperscript{14}. Although this device allowed for efficient sensitizer photorelease in octanol or other lipophilic media, photorelease in aqueous solution was inefficient due to readsorption of the hydrophobic dye onto the tip surface.

The discharge of the sensitizer from the probe tip is a key event in the method. To enhance the sensitizer release a third generation fiber optic system was developed with a fluorinated fiber tip\textsuperscript{18}. Fluoroalkylsilane coatings on porous silica have repellant and self-cleaning properties which enhanced the PPa cleavage efficiency by 15\%.

Additionally, improving the water solubility and reducing the aggregation propensity of the sensitizer itself, will contribute to its better bioavailability and an enhanced photochemical efficiency of \textsuperscript{1}O\textsubscript{2} production. To this end, we designed an improved probe tip for the photokilling of human ovarian cancer cells. This 4\textsuperscript{th} generation fiber optic delivery device uses PEGylated photosensitizer\textsuperscript{19} covalently attached onto PVG surface. Increasing numbers of PEG groups in the mono-, di-, and tri-PEG chlorin conjugates decreased self-aggregation, and increased the water solubility and sensitivity to hydrolysis and uptake into ovarian cancer cells.

As PDT efficiency depends on oxygen concentration as well as photosensitizer amount and light dosage, treatment of hypoxic tumors using this approach is complicated by low oxygen availability. Tumor hypoxia has been shown to limit the oxygen-dependent photosensitizer damage\textsuperscript{12,13}. Researchers have shown that oxygenation of tissue can enhance PDT efficiency\textsuperscript{19-21}, however, at present, there are no available means to oxygenate hypoxic tumor sites besides our fiber optic device\textsuperscript{19-21}. Fiber optic device delivers O\textsubscript{2} molecules due to the porous nature of the Vycor glass. Oxygen transmission through the probe tip into H\textsubscript{2}O solution occurs at a rate of 0.16
ppm/min at 10 PSI. A new fiber optic implement with increased oxygen permeability through the fiber tip could offer the advantage of better tissue oxygenation and solve the problem of hypoxia for tumor destruction due to the oxygen requirements for PDT.

To address the above mentioned issues and improve the efficiency of our PDT microoptic device for the photokilling of human cancer cells my work consist in designing a new biocompatible solid support for photosensitizer drug release. Our goal is to develop new Teflon based hydrophobic probe tip with (1) sensitiser repellent properties to increase sensitiser output (2) increase in ground-state oxygen solubility for a higher photocleavage efficiency and singlet oxygen production and (3) increase oxygen permeation for better tissue oxygenation.

1.6 References
5. Ravant, J.; Mascio, P.; Martinez, G.; Medeiros, M.; Cadet, J. Biol. Chem. 2000, 475, 40601-40604.


Chapter 2. Teflon-like Photoactive Surfaces that Release Sensitizer Drug Molecules Based on $^1$O$_2$ Reactions

2.1 Introduction. In 2015, a silica system was developed in our lab to release sensitizer molecules for the generation of $^1$O$_2$ in the immediate vicinity of the surface.$^{1-3}$ Delivery of PEGylated drugs is an area of much research.$^{4-9}$ The literature on PEGylated drugs is large,$^{4-9}$ and our recent work with PEGylated chlorin sensitizers reaffirm their usefulness in PDT. To date, there are few solids designed to deliver PEGylated sensitizer molecules. Because PEGylated compounds tend to adsorb to hydrophilic surfaces, their release from surfaces (e.g., silica) for a drug delivery strategy is challenging.

Solid supports have been used as platforms for the photorelease of drug molecules.$^{10-11}$ However, there are gaps in data on whether Teflon-like$^{12-13}$ or superhydrophobic surfaces$^{14}$ can efficiently photorelease drugs. To address this issue, two photoactive fluoropolymers have been synthesized and compared (Figure 3). One made of fluorinated silica [glass coated with (CH$_3$O)$_3$SiCH$_2$CH$_2$CF$_2$CF$_2$CF$_3$] (synthesized by my colleague Goutam Ghosh), and the other is a Teflon/poly(vinyl alcohol) (PVA) nanocomposite that has $-$[CF$_2$–CF$_2$]$_m$– and $-$[CH$_2$–CH(OH)]$_n$– chains. Figure 3 also shows that the sensitizer drug to be photoreleased is PEGylated. We also wondered whether the unwanted adhering of PEG sensitizers could be reduced with an appropriately designed solid support.

We have synthesized surfaces that are repellent (i.e. Teflon-like) to efficiently photorelease a PEGylated sensitizer drug to surrounding solution. Teflon is known as a biocompatible material (e.g., for surgery)$^{15-17}$ but not yet as a material for PEGylated drug release. The mechanistic information collected here is used to determine whether a Teflon/polyvinyl alcohol (PVA) polymer blend has an advantage to fluorinated silica.

While the delivery of PEGylated compounds is an active area of research,$^{18-20}$ they tend to adhere to surfaces.$^{21-23}$ Even though solid-state sensitizers have been established, few have been designed to release PEGylated compounds, and none have capitalized on fluoropolymers' nonstick repellent properties for better molecule discharge from the surface. Thus, we anticipated that fluoropolymer sensitizer release systems with repellent properties and visible light activation could be established.
Figure 3. Design of the surfaces tailored for $^{1}$O$_2$-initiated sensitizer drug photorelease reactions. The sensitizers, chlorin derivatives that were PEGylated with triethylene glycol, were bound to surface OH groups of (A) fluorinated silica (Vycor glass monolith coated with nonafluorosilane), and (B) a Teflon/polyvinyl alcohol nanocomposite. Red light irradiation leads to the attack of $^{1}$O$_2$ with the ethene resulting in the formation of a dioxetane. A second step follows with cleavage to release the sensitizer, although the sensitizer can adhere to the surface in an adsorbed state depending on the surface properties.

Visible light and NIR photocleavage reactions are known$^{24}$ and actually represent a burgeoning area of research.$^{25,26}$ For example, the sensitized generation of $^{1}$O$_2$($^{1}$Δg) has been used with labile ethene linkers for photorelease reactions. We$^{27,28}$ and others$^{29−31}$ have published papers devoted to $^{1}$O$_2$-based drug release, and a book chapter has also appeared.$^{32}$

Singlet oxygen is a potentially therapeutic species and is photogenerated by PAHs,$^{33,34}$ chlorins,$^{35,36}$ porphyrins and phthalocyanines, and their fluorinated analogues.$^{37−41}$ In 2011, Röder et al. reported the deposition of perfluorinated phthalocyanines on silica gel as a composite material for generating $^{1}$O$_2$ for sterilization.$^{42}$ A Teflon ponytail fullerene (i.e., C$_{60}$ adduct with CH$_2$(CO$_2$(CH$_2$)$_3$(CF$_2$)$_7$CF$_3$)$_2$) has also been prepared for $^{1}$O$_2$ generation.$^{43}$ Favorable properties of surface fluorination for $^{1}$O$_2$ and drug potency through PEGylation would make such a combination
be desirable. However, solid materials that are both fluorinated and PEGylated are rather uncommon.\textsuperscript{44} Taken together, the above topics reveal the potential utility of a Teflon supported PEGylated drug release system and point to the need for new studies.

Our hypothesis was that a Teflon/PVA nanocomposite will photorelease the PEGylated sensitizer more efficiently than fluorinated silica because of the higher number of C–F bonds in the former Fluorinated surfaces were selected for 3 reasons: (i) they show reduced adsorption to the PEGylated sensitizer drug, for better turnout; (ii) they increase the solubility of ground-state oxygen, which is advantageous for photooxidation chemistry to work well; (iii) the ethene bond is more labile where chemical quenching efficiency increases due to the C–F bonds that reduce physical quenching of singlet oxygen by the surfaces.

A “three-prong approach” will be used to assess the new fluorinated surfaces materials capable of releasing PDT concentrations of sensitizer drugs: (a) Repellent (non-sticky Teflon-like) fluorochemically modified materials for high biocompatibility will be synthesized. (b) An increase in ground-state oxygen solubility by the surface will be quantified. (c) The increase in the \(^1\)O\(_2\) and sensitizer output by the surface will be measured.

The outcome is establishing which design and coating is best for the probe tip to achieve \(^1\)O\(_2\) and sensitizer delivered directionally. We expect to achieve efficient singlet oxygen generation of the photosensitizer cleavage on-site by the fiber tip. Our mechanistic study provides results that may be useful for applications in localized delivery of sensitizer to desired surfaces (e.g., wounds or diseased tissue), where the released photosensitizer is active upon subsequent illumination.

2.2 Results

2.2.1 Fabrication and Post-Modification of Fluorinated Surfaces for Efficient Drug Photorelease. The new Teflon surface was prepared using a similar procedure reported by Avella\textsuperscript{45} by mixing a PTFE emulsion with PVA. Aqueous PVA solution was prepared first by dissolving PVA powder in distilled water at 90 °C for 5 h under constant stirring. The solution was cooled to room temperature and a Teflon suspension was added to the PVA solution in 6:1 Teflon/PVA mass ratio. The mixture was stirred at room temperature and atmospheric pressure for 2.5 h using a mechanical agitator. Samples were placed in a mold and dried at room temperature and atmospheric pressure for 1 week. FT-IR (cm\(^{-1}\)): 3600–3100, 2950–2850, 1419–1325, 1203, 1147. UV−vis (\(\lambda\), air): transparent.
The Teflon/PVA surface is a polymeric nanocomposite material of a PVA matrix filled with Teflon nanospheres.\textsuperscript{45} The surfactant provided a means for the Teflon nanoparticles to be evenly dispersed into the PVA matrix. We did no observe clusters of Teflon particles. It consists of a smooth, PVA phase with high content of submicron Teflon (22.3 nm) particles: a PVA polymeric matrix filled with PTFE nanospheres. The surfactant allowed PTFE nanoparticles to be evenly dispersed into the PVA matrix, no clusters of PTFE particles were observed. Dispersion of PTFE into the polymeric matrix and drying was done at room temperature which ensured the presence of PVA into the material. Heat during synthetic process and drying above 340 °C causes PVA decomposition leaving behind a porous PTFE material without PVA.\textsuperscript{46} This kind of porous PTFE material (without PVA) is not desired because would exclude the possibility of surface functionalization. We did not use the high temperatures and so the Teflon /PVA nanocomposite film was formed and we can react on the PVA alcohol regions.

To prepare the succinic acid functionalized Teflon/PVA surface succinic acid (0.100 g, 8.5 mmol) was dissolved in 1 mL anhydrous dimethylformamide (DMF) and added to five pieces (ea. 0.22 g) of Teflon/PVA in 15 mL anhydrous CH\textsubscript{2}Cl\textsubscript{2} then EDC (0.081 g, 0.425 mmol) and DMAP (0.052 g, 0.425 mmol) were added. The reaction was stirred under nitrogen atmosphere for 5 days. The samples were washed with solvents (CH\textsubscript{2}Cl\textsubscript{2}, THF, methanol, toluene, and hexane) and Soxhlet extracted with methanol for 24 h to remove any unreacted, adsorbed succinic acid. FTIR confirmed the covalent attachment of Succinic acid onto the Teflon/PVA surface. FT-IR (cm\textsuperscript{-1}): 1723. UV–vis (\(\lambda\),air): transparent.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figures/figure4.png}
\caption{Synthesis of spacer triPEG chlorin 5.}
\end{figure}
The sensitizer we used for drug attachment was spacer triPEG chlorin e₆. Synthesis of spacer triPEG chlorin sensitizer was done by my colleague Dr. Goutam Ghosh and the synthetic steps are shown in Figure 4.

To prepare the triPEG chlorin-modified Teflon/PVA the succinic acid functionalized Teflon/PVA tips (each piece was 0.21 g and sized ~4 mm × ~7 mm (d × l)) were placed in a 10 mL CH₂Cl₂ with 6 mg chlorin e₆ triPEG 5 (4.3 µmol), 0.010 g EDC (0.052 mmol) and 0.010 g (0.082 mmol) DMAP. The reaction mixture was stirred under nitrogen at room temperature for 5 days. After reaction the samples were washed with several solvents (CH₂Cl₂, THF, methanol, toluene, hexane) and Soxhlet extracted using methanol for 24 h. FT-IR (cm⁻¹): 1731. UV–vis (λ, air): 396 and 660 nm.

Figure 5. Synthesis of sensitizer-conjugated organically to Teflon/PVA nanocomposite 10 and by-product formation of surface diester sites.

The amount of sensitizer attached onto the succinic acid sites of Teflon/PVA was determined to be 23.4 nmol/g by UV–vis spectroscopy. UV–vis (λ, air): 396 and 660 nm. IR data were unable to distinguish whether the excess EDC used converted many of the surface acid sites to diesters. Teflon/PVA 10 was stable in the dark; the sensitizer did not leach out to any measurable extent after CH₂Cl₂, methanol, THF, and hexane solvent washings or Soxhlet extraction with methanol.
Synthetic steps of the sensitizer-conjugated fluorinated silica are shown in Figure 6. Sensitizer conjugated-fluorinated PVG surface was synthesized by my colleague Goutam Ghosh. For both surfaces the sensitizers, chlorin derivatives that were PEGylated with triethylene glycol, were bound to surface OH groups of (A) fluorinated silica (Vycor glass monolith coated with nonafluorosilane), and (B) a Teflon/polyvinyl alcohol nanocomposite (Figure 4).

2.2.2 PEGylated Sensitizer Photorelease from the Fluorinated Solid Supports. The photorelease efficiency for sensitizer was assessed in O2-saturated n-butanol solutions by irradiation with a 669-nm diode laser. The photolysis setup included a continuous wave diode laser where the light was passed through an SMA port and out of the end of a borosilicate optical fiber with SMA optical fiber coupling, as has been described in our previous work. Under subdued light, n-butanol solutions were presaturated with O2 for 20 min and then illuminated with red light for 1.5 h, upon which 1O2 was generated and trapped by the alkene sites on the solid supports. These heterogeneous photolysis reactions contained 0.328 and 0.214 g of solids 7 and 10, respectively. Concentrations of 13 were measured based on calibration curves, which followed its Q-band absorption at 665 nm. The number of broken alkene bond was quantified by the amount of sensitizer detected in after Soxhlet extraction in methanol. Control experiments demonstrated that the photorelease does not deviate from Beer’s law in the UV–vis detection of sensitizer. Compound 13: HRMS (+ESI) m/z calcd for C_{66}H_{89}N_{4}O_{21} [M + H]^+, 1273.6014; found, 1273.6018. UV–vis (CHCl3) λ_{max} 399 and 660 nm. No other products were found in solution based on GC/MS and NMR spectroscopy.
Figure 7. Photooxidation of fluorinated silica 7 and Teflon/PVA nanocomposite 10 leads to the photorelease of sensitizer 13.

Table 1. Yield of Photorelease by Teflon/PVA and Fluorinated Silica Photooxidation$^{a,b}$

<table>
<thead>
<tr>
<th>Solid Supports</th>
<th>Photoreleased 13 (nmol)</th>
<th>Photoreleased 13 (%)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teflon/PVA</td>
<td>0.87±0.04</td>
<td>17.37±0.93</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1.25±0.11</td>
<td>25.03±2.20</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1.48±0.03</td>
<td>29.67±0.50</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1.48±0.03</td>
<td>29.67±0.50</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1.48±0.03</td>
<td>29.67±0.50</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>1.48±0.03</td>
<td>29.67±0.50</td>
<td>90</td>
</tr>
<tr>
<td>Vycor</td>
<td>1.34±0.05</td>
<td>4.46±0.20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2.57±0.20</td>
<td>8.58±0.73</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3.35±0.31</td>
<td>11.14±1.05</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>3.76±0.14</td>
<td>12.54±0.48</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>4.10±0.23</td>
<td>13.72±0.73</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>4.17±0.33</td>
<td>13.97±1.08</td>
<td>90</td>
</tr>
</tbody>
</table>
aExternal irradiation of tip via a fiber optic connected to 669 nm diode laser and operated at 4.0 psi O₂ pressure, 0.2-0.3 ppm/min O₂ flow rate through the 0.328 g fluorinated silica and 0.214 g Teflon/PVA probe tip. Fiber tip dimensions: cylinder shape with a length of 8.0 mm, diameter of 5.0 mm, and hole (2.0 length x 3.0 mm diameter) for F-PVG and cylinder shape with a length of 7.0 mm, diameter of 4.0 mm, and hole (2.0 length x 3.0 mm diameter) for Teflon/PVA surface. Experiments were repeated thrice. bAbsorption spectroscopy was used for the quantitation of sensitizer in n-butanol.

Table 1 and Figure 8 show that the percent PEGylated sensitizer photorelease in n-butanol was higher for the Teflon/PVA surface than for fluorinated silica surface. We found ~ 15 % and ~ 30 % photorelease after 90 min in n-butanol for 89.4 nmol and 21.7 nmol loaded triPEG per gram of fluorinated silica and Teflon/PVA probe tip respectively.

Figure 8. Evolution of the sensitizer 13 which photo-departed away from the fluorinated silica 7 (●) and Teflon/PVA 9 (■) into n-butanol solution at 25 °C. Error bars represent the standard deviation obtained from 3 measurements.

The emergence of sensitizer 13 in n-butanol was quantified by monitoring its Q–band absorption by UV–vis. Control experiments show that the red light and O₂ were needed to cause the photorelease of 13. Control experiments also showed that the ester groups of 7, 10, and 13 remained intact under dark reaction conditions. We did not find any evidence for ester bond
hydrolysis under the conditions where all sensitizer release was due to reaction of $^1$O$_2$ with the ethene sites. In terms of the ethene linker bonds broken, a greater percent release of sensitizer occurred for Teflon/PVA surface compared to fluorinated PVG. Although the quantity of sensitizer loaded on fluorinated PVG was greater than Teflon surface.

To address the adsorptive affinity of PEGylated sensitizer for the fluorinated surfaces, further experiments were carried out, as will be described in the next section. Amount of adsorbed sensitizer, which didn’t come off after probe-tip photooxidation, was quantified by 24 h of Soxhlet extraction of the photooxidized probe-tips in methanol and measuring concentration of sensitizer in methanol by UV-vis spectroscopy which is shown in Table 2. Adsorption of sensitizer to the fluorinated silica and Teflon/PVA probe-tip was 48.4 nmoles and 13.4 nmoles respectively. Amount photocleavage (broken alkene bond) was quantified by addition of the amount of photoreleased sensitizer in $n$-butanol and sensitizer in Soxhlet extracted methanol. For fluorinated silica, we found a total of 68.3% of sensitizer photocleaved upon photochemical reaction, whereas for Teflon/PVA it was 91.7%.

**Table 2. Photorelease of Sensitizer on Fluorinated Surfaces.$^{a,b}$**

<table>
<thead>
<tr>
<th>Solid support</th>
<th>Sens loading (nmol)</th>
<th>Sens 13 photocleaved (nmol)</th>
<th>% sens photocleaved</th>
<th>Sens adsorbed (nmoles)</th>
<th>Ethene linker bonds broken (nmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorinated silica 7</td>
<td>89.4</td>
<td>12.7</td>
<td>68.3</td>
<td>48.4</td>
<td>61.1</td>
</tr>
<tr>
<td>Teflon/PVA 10</td>
<td>21.7</td>
<td>6.5</td>
<td>91.7</td>
<td>13.4</td>
<td>19.9</td>
</tr>
</tbody>
</table>

$^{a}$ Red light from a diode laser was used to photocleave the sensitizer. Absorption spectroscopy was used to quantitate the amount of sensitizer in the surrounding $n$-butanol solution.$^{b}$ The data show weights for 7 and 10 normalized to 1.0 g.

Number of sites for sensitizer to be covalently bound on SiOH (1.3x10$^{20}$) of F-SiO$_2$ surface was 7 fold greater than –COOH (3.05x10$^{-5}$) of Teflon/PVA surface. We loaded only 0.023 μmol sensitizer per gram of cap when 4.3 μmol of sensitizer was used as a starting material, which is only 0.5% yield. It indicates that significant amount of surface succinic acid (~99.5%) is forming diester with the -CH-OH on the surface shown in Figure 5. Loading of triPEG to the Teflon/PVA polymer was about 4 fold lower than to the fluorinated Vycor. Lower loading to the Teflon/PVA may be caused by not having significant amount of available carboxylic acid group on the surface.
to covalently link the sensitizer. In the succinic acid monocarboxylate-Teflon/PVA, one of the carboxylic acid is no longer available for esterification with spacer alkene alcohol moiety, when in presence of EDC-DMAP, succinic acid monocarboxylate forms bis-ester with the surface hydroxyl groups.

A reason for lower photocleavage of sensitizer from sensitizer conjugated fluorinated silica is that fluorinated silica surface is 47 fold more crowded compared to the succinic acid modified Teflon/PVA, when we compared between amount of pendant nonafluorohexyl silane and succinic acid on the fluorinated silica and succinate-Teflon/PVA respectively. Crowding of the moiety may prevent the facile approach of singlet oxygen to the alkene site; thereby reduce the amount photocleavage, which occur by formation of dioxetane intermediate. The ethene bonds of the fluorinated silica sensitizer are stable to boiling methanol and toluene over many hours.

In terms of loading as mentioned above, the quantity of sensitizer loaded on fluorinated PVG surface was higher than that onto Teflon surface. High loading of sensitizer molecules is undesirable, especially when porphyrin sites are within the Foster radius that produces self-quenching for a photothermal rather than a photosensitized polymer. This led us to further analyze the number and type of functional groups (table 3) on our fabricated surfaces for insight into the mechanisms and origins of sensitizer photorelease upon fluorinated PVG or Teflon/PVA surface photooxidation.

Table 3. Absolute number of bonds or functional groups on the fabricated surfaces per gram

<table>
<thead>
<tr>
<th>solid support</th>
<th>Si−OH</th>
<th>C−OH</th>
<th>C−H</th>
<th>C−F</th>
<th>sens</th>
<th>C−OH:</th>
<th>C−F:sens</th>
<th>Si−OH:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fluorinated silica 7</td>
<td>1.3×10²⁰</td>
<td>-</td>
<td>3.5×10²¹</td>
<td>7.8×10²¹</td>
<td>5.5×10¹⁶</td>
<td>-</td>
<td>2400:142000:1</td>
<td></td>
</tr>
<tr>
<td>Teflon/PVA 10</td>
<td>-</td>
<td>6.2×10²⁰</td>
<td>1.9×10²¹</td>
<td>9.6×10²¹</td>
<td>1.3×10¹⁶</td>
<td>48000:750000:1</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

a Fluorinated silica 7 contains pendent nonafluorosilane groups and the Teflon/PVA nanocomposite contains repeating –[CF₂-CF₂]ₘ– and –[CH₂-CH(OH)]ₙ– units. The penetration depth of sensitizer into 7 was 0.08 mm and into 9 was 1.0 mm.

Table 3 shows the calculated quantity of C−F, C−H, C−OH and Si−OH bonds in fluorinated silica and Teflon/PVA nanocomposite. The number of O−H bonds is important to tabulate not only
because they can serve as adsorption sites. They also offer more efficient radiationless deactivation\textsuperscript{49,50} of $^1$O\textsubscript{2} by electronic to vibrational energy transfer\textsuperscript{51} which follows the order: O–H > C–H > C–F. The number of O–H bonds for 10 is 4.8 fold greater than in 7. Also for 10 compared to 7, there is a 1.2-fold more C–F bonds and 1.8-fold less C–H bonds. There is a greater number of sites for sensitizer to be covalently bound on Si–OH ($1.3\times10^{20}$) of fluorinated PVG than CH–OH ($6.2\times10^{20}$) of Teflon/PVA, which supports the observation that the 6-fold higher loading of sensitizer on silica surface compared to Teflon surface.

For sensitizer conjugated Teflon/PVA surface, 0.5 % coverage of sensitizer is observed, where a significant amount of surface succinic acid (~99.5 %) remained on the CH–OH portion of the surface. Reduced physical quenching of $^1$O\textsubscript{2} by the surface can be by control by adjusting the spatial distance between the sensitizer molecules. Overloading will convert the system to a photothermal material instead of a sensitizer surface. The greater number of O–H sites on than Teflon/PVA surface then on fluorinated PVG led us to study the adsorptive affinity of sensitizer PEGylated sensitizer to the fluorinated surfaces.

\textbf{2.2.3 Testing the Fluorinated Surfaces for Resistance to Sensitizer Drug Adsorption.} We wondered what levels of unwanted adhering of PEG sensitizer would occur with our fluoropolymer supports. Figure 9 shows the amount of sensitizer that adsorbs onto fluoropolymers (here, sensitizer less 6 and 9 were examined) placed in a 10 μM n-butanol solution of sensitizer chlorin e\textsubscript{6}-trPEGester. Experiments were carried out with cylindrical pieces of fluorinated silica, Teflon/PVA and succinic acid functionalized Teflon/PVA (0.32 g) immersed in n-butanol solutions of chlorin 13 for 0.5, 1.0, and 1.5 h and to analyze the level of adsorption that occurs on the surface. Figure 10 shows the ability of the fluorinated surfaces to resist the adsorption of PEGylated sensitizer.
Figure 9. Effect of surface type on the adsorption of sensitizer. Adsorption profile of sensitizer (initial concentration of chlorin e₆-triPEG ester 10 μM). % Adsorption of dye (chlorin e₆-triPEG ester) to the fluorinated Vycor (lighter grey bar), succinic acid functionalized Teflon/PVA (darker grey bar) and Teflon/PVA (black bar) surfaces were studied for 90 min. 27% dye adsorption for fluorinated Vycor was found, whereas only 1.25% adsorption occurred for Teflon/PVA polymer after 90 min.

Figure 10 and table 4 show that the adsorptive capacity of the PEGylated sensitizer increased significantly with succinic acid-Teflon/PVA surface. 41.5% of the PEGylated sensitizer was adsorbed onto its surface in 90 minutes. Sensitizer adsorption to the fluorinated surfaces was reduced and a 27.06% adsorption was observed for the Fluorinated PVG and only 1.25% adsorption for the Teflon/PVA surfaces. The sensitizer adsorbed onto Teflon/PVA and fluorinated silica did not desorb unless washed with solvents (methanol, dichloromethane) and Soxhlet extracted with methanol.

The sensitizer’s ability to adsorb to the surfaces was increased in the order Teflon/PVA > fluorinated silica > succinic acid-Teflon/PVA. This can be understood due to the tendency of PEGylated compounds to adhere to surfaces with OH groups. This result is somewhat similar with our recent work (Bartusik 2012) where pheophorbide sensitizers increased in its adsorptive affinity for silica with higher quantities of O-H groups. Clearly, the sensitizer forms a weaker adsorbate based on lower quantities of hydrophilics in plain Teflon/PVA than fluorinated PVG or in particular owing to the succinic acid sites in SA modified Teflon/PVA.
**Figure 10.** Percent of PEGylated sensitizer adsorbed onto fluorinated surfaces [fluorinated silica 7 (▲), Teflon/PVA 8 (■), and succinic acid functionalized Teflon-PVA 9 (●)] in n-butanol. The samples were immersed in n-butanol, which contained 10 µM sensitizer and then samples were removed at the indicated times.

**Table 4.** Adsorption of triPEG in n-butanol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fluorinated Vycor nmol (%)</th>
<th>Teflon/PVA nmol (%)</th>
<th>SA-Teflon/PVA nmol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>30</td>
<td>3.62 (12.06 %)</td>
<td>0.14 (0.44 %)</td>
<td>5.88 (19.6 %)</td>
</tr>
<tr>
<td>60</td>
<td>6.68 (22.27 %)</td>
<td>0.27 (0.84 %)</td>
<td>9.36 (31.2 %)</td>
</tr>
<tr>
<td>90</td>
<td>8.12 (27.06 %)</td>
<td>0.40 (1.25 %)</td>
<td>12.46 (41.5 %)</td>
</tr>
</tbody>
</table>

*aAdsorption of chlorin e₆-triPEG ester onto Fluorinated Vycor, SA-Teflon/PVA (Succinate-Teflon/PVA) and Teflon/PVA were measured. Q band adsorption at 664 nm was followed. The samples were immersed in n-butanol, which contained chlorin e₆-triPEGester (10.0 µM). The samples were removed at the indicated times.

**Lifetime Measurements.** (Measurements were done by my colleague Inna Abramova and Professor Greer). Table 5 shows that the total rate constants (k_T) of ^1^O_2 decreased in the presence of fluorinated silica, Teflon/PVA, and Teflon particles compared to that with native silica. In these
experiments, the 1270 nm signal of $^1\text{O}_2$ was followed with added quantities of the solid particles in acetone-$h_6$ at 25 °C. Native silica, 6, 8, and Teflon were used because they lacked the sensitizer heads of 7 and 10, where a constant concentration of rose Bengal was used in the heterogeneous mixtures.

Teflon, 6, and 8 contain a high number of C−F oscillators, which do not efficiently physically quench $^1\text{O}_2$ compared to that by C−H or O−H oscillators due to electronic to vibronic overlap. Consistent with this notion, for native silica, the $k_T$ value increased by 2−3-fold because of the greater numbers of O−H bonds. The poor $R^2$ values in entries 3 and 4 (Table 4) are not a cause for concern where Teflon/PVA 8 and Teflon were ground to flakes that were larger and harder to stir as a slurry than the silica particles. Clearly, the data show success in using fluorinated surfaces due to their reduced physical quenching of $^1\text{O}_2$. The low $k_T$ values we observe are encouraging since less wasted $^1\text{O}_2$ by the fluorinated surface is vital to its success for drug photorelease.

Table 5. Rates of Reaction with Singlet Oxygen by Native Silica, Fluorinated Silica 6, Teflon/PVA 8, and Teflon in Acetone

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>$k_T$ (L g$^{-1}$ s$^{-1}$)</th>
<th>$R$ square</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Native silica</td>
<td>71±6</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>Fluorinated silica 6</td>
<td>27±3</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>Teflon/PVA 8</td>
<td>29±9</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>Teflon</td>
<td>10±8</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*Heterogeneous mixture of particles (75−150 μm sized silica and 300−400 μm sized Teflon) in acetone-$h_6$ at 25 °C. Average of 2−3 experiments.

2.2.4 Dioxigen Solubility Enhancements Caused by the Fluorinated Surfaces. We evaluated the effect of the fluorinated (Teflon/PVA polymers and fluorinated silica) and nonfluorinated materials (native PVG) on the $O_2$-carrying capacity in butanol. The hypothesis was that a fluorinated surface will yield high oxygen concentrations consequently producing higher quantities of singlet oxygen. A $pO_2$ electrode was used to measure the level of $O_2$-carrying capacity due to enhanced molecular interactions of $O_2$ based on fluorination ratio and conditioning of the
probe tip surfaces. A minimum of 0.1 mM O₂-solubility increase in water solution and cell suspensions is desired based on examples of O₂ concentration increases in fluorinated media, such as perfluorinated artificial blood.

The 0.58 g solid sample (PVA/ Native silica/ Teflon/PVA coated with SA / Teflon-PVA/ Fluorinated silica) was placed into a three neck RBF flask and 5mL of butanol was added. The oxygen meter probe was placed inside the RBF and the flask was sealed. Oxygen was purged from an oxygen tank into the flask with a flow of ~ 70 ml/min O₂. Concentration of oxygen in butanol was recorded after 20 min of O₂ purging.

Table 6. Oxygen Solubility Enhanced by Fluorinated Materials in Solution

<table>
<thead>
<tr>
<th>Solid</th>
<th>ppm</th>
<th>mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native silica</td>
<td>14.9±0.45</td>
<td>0.466±0.015</td>
</tr>
<tr>
<td>Fluorinated silica 6</td>
<td>16.7±0.39</td>
<td>0.522±0.013</td>
</tr>
<tr>
<td>PVA</td>
<td>14.9±0.03</td>
<td>0.466±0.001</td>
</tr>
<tr>
<td>Teflon/PVA nanocomposite 8</td>
<td>17.9±0.07</td>
<td>0.559±0.003</td>
</tr>
</tbody>
</table>

aPyrex test tubes contained 5.0 mL of O₂-saturated n-butanol with 0.58 g of solid at 25 °C. A pO₂ electrode was used to measure the O₂ solubility. After removing the solids from solution, the O₂ solubility returns to 14.9 ppm (0.466 mM).

Table 6 shows that the O₂ solubility increases in the presence of the fluorinated materials (fluorinated silica and Teflon/PVA nanocomposite) compared to that with native PVA and silica, where a 1.2 ppm increase is shown. We found that addition of 0.58 g of fluorinated Vyvor enhances oxygen O₂ solubility to 16.7 ppm (0.522 mM) in n-butanol. Whereas, addition of same amount of Teflon/PVA or SA coated Teflon/PVA polymer increased O₂ solubility to 17.8 (0.556 mM) and 17.9 ppm (0.559 mM) respectively. O₂ solubility increases in fluorous media has been observed previously in fluorinated artificial blood, ionic liquids, solvents, and biological systems. As will be evident, the fluorinated solids provide an opportunity to enhance the production of O₂ due to higher local O₂ concentrations.
2.2.5 Mechanism of Sensitizer Photorelease from the Teflon-like Surfaces.

There is enhanced O\textsubscript{2} solubility with our fluoropolymers compared to that in nonfluorinated media, so more \( ^1\text{O}_2 \) can be generated. Fluoropolymer surface topology likely relates to O\textsubscript{2} solubility increases based on previous NMR studies,\textsuperscript{61} where shapes of perfluoro sites and the existence of cavities in the liquid phase dominate over any direct interactions of O\textsubscript{2} with the fluorine atoms, such as charge-transfer interactions.

Singlet oxygen is generated by surfaces \textit{7} and \textit{10}, mainly from surface triplet sensitizer\textsuperscript{*} quenching by O\textsubscript{2} that leads to a dioxetane. We know that the dioxetane is not stable\textsuperscript{62,63} and spontaneously breaks apart at room temperature, so the covalent bond between the sensitizer and the surface is lost. As yet, increased kinetic persistence of dioxetanes through sterics, as has been noted in adamantaneadamantylidene dioxetane which is stable at room temperature,\textsuperscript{64} cannot be attributed to our fluoropolymers. But, compare parts A and B of Figure 11, where the fluorine groups are oriented differently.

Because fluorinated silica \textit{7} consists of branched fluorosilane (high aspect ratio) groups, it is more subject to dynamical motion,\textsuperscript{65} whereas Teflon/PVA \textit{10} consists of continuous endon carbon–fluorine chains in which Teflon –[CF\(_2–CF_2\)]\(_m\)– may also coil into a helix due to repulsion between vicinal fluorine atoms.\textsuperscript{66}

The importance of steric interactions of the fluorosilane is consistent with our observation of reduced photocleavage efficiency in \textit{7} compared to that in \textit{10}, but this is confounded by factors such as repelling and quenching (described below). However, it seems obvious that crowding by branched fluorosilanes can prevent \( ^1\text{O}_2 \) accessibility to the ethene site. Indeed, dioxetane experiments come to mind that can probe mechanical,\textsuperscript{67} cage and hemiluminescence,\textsuperscript{68–69} and dynamical features of the \( \sim 1.2 \) nm length fluorosilane, i.e., nanoheterogeneity, of \textit{7} and \textit{10}, but these are beyond the scope of the present study.
After the ethene is photooxidized and broken, there is a partition between surface release and adsorption channels. The shuttling or mobility of the sensitizer on the surface prior to departure was not scrutinized with our fluoropolymers. However, the small decrease in the adsorptive affinity of 13 for 10 compared to that for 7 is attributed to the higher number of surface O–H groups in 10 relative to 7. However, does the adsorptive affinity interfere with further ethene photooxidation? Tying up some of sensitizer 13 in an adsorbed state means that autocatalytic-assisted release kinetics are unavailable, unlike that for more repellent hydrophobic sensitziers.

For our fluoropolymers 7 and 10, the ethene sites are photooxidized. The data point to fluorinated media and an increase in $\tau\Delta$ due to inefficient radiationless deactivation of $^{1}\text{O}_2$ by C–F bonds compared to O–H and C–H bonds. Unlike polyfunctionalized compounds or proteins, the reaction center for 7 and 10 is the ethene site. Other than the ethene site, the surfaces do not chemically react with $^{1}\text{O}_2$, as would be expected for Teflon-like materials that are...
known to resist oxidation. We find the PEG groups of 7 and 10 were not susceptible to photooxidation. This is not the case for oxygen radicals, e.g., via autoxidation, where PEG hydroperoxides can form and degrade.

In summary, we find that the presence of C–F bonds in these materials leads to efficient photocleavage of the PEGylated sensitizer. Teflon/PVA provides a slight advantage with respect to the sensitizer photorelease application compared to that with fluorinated silica.

2.2.6 Additional Studies on the PEGylated Sensitizer-Teflon/PVA Surface. As described in the above section, we have been successful in covalently attaching and photocleaving from the Teflon/PVA surface a highly cytotoxic drug to ovarian cancer cell, chlorin e₆-triPEG. The system used external visible light to cleave the photosensitizer away from the Teflon/PVA surface. Our next goal was to prepare a new chlorin e₆-triPEG-Teflon surface, couple it with our microoptic PDT device, and then assess sensitizer photocleavage efficiency from the surface upon internal irradiation.

A new sensitizer-Teflon/PVA surface was synthesized using a similar strategy for covalent attachment as shown in Figure 5 and experimental procedure described in section 2.2.1. To prepare the triPEG-chlorin-Teflon/PVA the succinic acid functionalized Teflon/PVA (3 pieces) were added to 10 mL CH₂Cl₂ with 12 mg chlorin e₆ triPEG (8.6 µmol), 0.015 g EDC (0.078 mmol) and 0.010 g (0.082 mmol) DMAP. The reaction mixture was stirred under nitrogen at room temperature for 5 days. After reaction the samples were washed with several solvents (CH₂Cl₂, THF, methanol, toluene, hexane) and Soxhlet extracted using methanol for 24 h. FT-IR (cm⁻¹): 1731. UV-vis (λ, air): 396 and 660 nm.

The amount of chlorin-tri-PEG attached onto the succinic acid functionalized Teflon/PVA surface was determined by spectrophotometry. UV of the reaction mixture was taken before and after attachment. The Soret band was followed at 400 nm and the UV spectra can be seen in Figure 12. The amount of chlorin-tri-PEG attached per 1 g Teflon/PVA was 160 nmoles.
Figure 12. UV absorption spectra of reaction mixture containing chlorin-triPEG before (black) and after reaction (red).

Surface morphological changes of the plain Teflon/PVA and triPEG-chlorin-modified Teflon/PVA were investigated by SEM operating at 10kV at the magnification of 1720X and 20000X. As can be seen from Figure 13 the unmodified Teflon/PVA surface consists of a smooth, PVA phase with high content of submicron PTFE particles, seen at 20000X magnification in the image on the right.

Figure 13. SEM image at 1720X (left) and 20000X (right) magnification of plain Teflon/PVA before sensitizer covalent attachment showing a smooth PVA surface with high content of submicron PTFE particles.
Figure 14. SEM image at 1720X (left) and 20000X (right) magnification of chlorin e6-Teflon/PVA surface showing changes in surface morphology upon sensitizer attachment: presence of aggregates on the polymer surface (left image) and an increase in surface area and groves formation (right image).

Upon sensitizer and succinic acid covalent attachment we observed that Teflon/PVA surface morphology changed, see Figure 14. The surface structure seen at 1720X magnification shows the appearance of aggregates on the polymer surface (Fig. 14, left) while the surface structure seen at 10000X shows that there is an increase in surface area and groves formation (Fig. 14, right). The aggregates appeared to the Teflon/PVA functionalized surface, as seen in Figure 14, confirm sensitizer and succinic acid presence on the surface.

Photocleavage efficiency of the covalently attached chlorin e6-SA-triPEG from the probe tip surface coupled with the microoptic PDT device was also determined. 0.250 g Teflon/PVA hybrid tip, previously loaded with 40 nmoles dye conjugate, was affixed to the hollow fiber optic, where O2 flowed and diode laser light was delivered through the tip and place in 700 µl n-butanol. Sample was irradiated with a continuous wave diode laser (669 nm, 506 mW, 2.5A output) connected to a custom made fiber optic cable whose distal end had a stainless still ring where the Teflon/PVA cap was glued with ethyl cyanoacrylate. The laser was connected to the proximal end of the fiber through an SAM connector. The fiber optic used was 3ft in length and had a Teflon gas flow tube running from the distal end to a T valve. Gas flowed from a compressed oxygen tank and subsequently to the T-valve in the hollow fiber. The O2 pressure was 3 PSI.

Sample was irradiated for 105 minutes and UV absorbance was measured every 15 minutes (Figure 15 and Table 7). Soret absorption band was monitored at 660nm and the amount of dye photocleaved was determined from the calibration plot in n-butanol.
Figure 15. Time course of photorelease of chlorin e₆-triPEG in 1-butanol solution arising from photooxidative cleavage and departure from the fiber optic device tip. The absorption spectra show the Q band at 660 nm. Dye loading was 40 nmoles per 0.250 g cap.

Table 7. Percent of sensitizer photorelease from the Teflon/PVA surface in butanol. The 0.250 cap was loaded with 40 nmoles.

<table>
<thead>
<tr>
<th>Time</th>
<th>Absorbance</th>
<th>Amount photocleaved in nmoles</th>
<th>Percent photocleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>0.540</td>
<td>12.53</td>
<td>31.33 %</td>
</tr>
<tr>
<td>30 min</td>
<td>0.644</td>
<td>14.94</td>
<td>37.35 %</td>
</tr>
<tr>
<td>45 min</td>
<td>0.729</td>
<td>16.91</td>
<td>42.27 %</td>
</tr>
<tr>
<td>60 min</td>
<td>0.756</td>
<td>17.53</td>
<td>43.83 %</td>
</tr>
<tr>
<td>75 min</td>
<td>0.778</td>
<td>18.05</td>
<td>45.13 %</td>
</tr>
<tr>
<td>90 min</td>
<td>0.778</td>
<td>18.05</td>
<td>45.13 %</td>
</tr>
<tr>
<td>105 min</td>
<td>0.778</td>
<td>18.05</td>
<td>45.13 %</td>
</tr>
</tbody>
</table>
Figure 16. Percent of chlorin-triPEG photorelease from the Teflon/PVA surface in 1-butanol with internal irradiation.

Using internal irradiation of the heterogeneous probe tip coupled to the fiber optic we found that the ethane spacer was photooxidized and led to 45.1% chlorin e₆-triPEG photorelease in 1-butanol after 75min (Figure 16 and Table 7). 17.5 nmol (25 µM) dye was photocleaved into n-butanol after 75 min (Table 8) when 40 nmoles of chlorin e₆-triPEG was loaded per 0.250 g Teflon/PVA cap. A control experiment was done in the absence of light and no cleavage of sensitizer molecule from the surface was observed in butanol.

Table 8. Amount of sensitizer loaded and photorelease in 75 min from Teflon/PVA surface.

<table>
<thead>
<tr>
<th>Loading/Cap</th>
<th>Time</th>
<th>Photocleavage (nmol)</th>
<th>Photocleavage (µM)</th>
<th>Photocleavage efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 nmoles/0.250 g cap</td>
<td>75 min</td>
<td>17.5 nmol</td>
<td>25</td>
<td>45.1%</td>
</tr>
</tbody>
</table>

We wanted to continue our surface adsorption studies by assessing the Teflon/PVA resistance to drug adsorption using a model compound, chlorin e₆-trimethylene. Its structure is shown in Figure 17. The Teflon/PVA surface resistance to drug adsorption was tested in both dichloromethane and 1-butanol.
Figure 17. Chemical structure of chlorin e₆-trimethylster.

Table 9. Nanomoles and percent adsorption of chlorin e₆-trimethylster onto native Vycor, Fluorinated Vycor and Teflon/PVA surfaces in dichloromethane.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Native Vycor nmoles (and %) adsorbed</th>
<th>Fluorinated Vycor nmoles (and %) adsorbed</th>
<th>Teflon/PVA nmoles (and %) adsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 nmoles (0%)</td>
<td>0 nmoles (0%)</td>
<td>0 nmoles (0%)</td>
</tr>
<tr>
<td>30</td>
<td>11.4 nmoles (23.8 %)</td>
<td>8.2 nmoles (17.1 %)</td>
<td>0.38 nmoles (0.8 %)</td>
</tr>
<tr>
<td>60</td>
<td>17.5 nmoles (36.5 %)</td>
<td>12.4 nmoles (25.8 %)</td>
<td>0.50 nmoles (1.0 %)</td>
</tr>
<tr>
<td>90</td>
<td>22.5 nmoles (46.9 %)</td>
<td>12.9 nmoles (26.9 %)</td>
<td>0.60 nmoles (1.2 %)</td>
</tr>
<tr>
<td>120</td>
<td>27.9 nmoles (58.1 %)</td>
<td>13.0 nmoles (27.1 %)</td>
<td>0.65 nmoles (1.3 %)</td>
</tr>
<tr>
<td>150</td>
<td>30.3 nmoles (63.1 %)</td>
<td>16.1 nmoles (33.5 %)</td>
<td>0.65 nmoles (1.3 %)</td>
</tr>
<tr>
<td>180</td>
<td>33.4 nmoles (69.6 %)</td>
<td>16.6 nmoles (34.6 %)</td>
<td>0.65 nmoles (1.3 %)</td>
</tr>
<tr>
<td>210</td>
<td>34.6 nmoles (72.1 %)</td>
<td>17.1 nmoles (35.6 %)</td>
<td>0.66 nmoles (1.4 %)</td>
</tr>
<tr>
<td>240</td>
<td>36.6 nmoles (76.3 %)</td>
<td>18.0 nmoles (37.5 %)</td>
<td>0.66 nmoles (1.4 %)</td>
</tr>
</tbody>
</table>
Adsorption of chlorin e\textsubscript{6}-trimethylester in dichloromethane onto native Vycor, Fluorinated Vycor and Teflon/PVA surfaces samples, was monitored by UV-vis spectrophotometry, followed at \( \lambda = 403 \text{nm} \). The samples were immersed in dichloromethane (5mL), which contained chlorin e\textsubscript{6}-trimethylester (0.96x10\textsuperscript{-5}M). The samples were removed at the indicated time.

Figure 18. Adsorption of chlorin e\textsubscript{6}-trimethylester at \( t=0 \) s (top) and \( t=3 \) h (bottom) onto native Vycor (left), fluorinated Vycor (middle) and Teflon/PVA (right) after 3 h.

The ability of native Vycor, fluorinated Vycor and Teflon/PVA to resist drug absorption is shown in Table 9 and Figure 18. It was observed that fluorinated surfaces have an increased resistance to drug adsorption vs nonfluorinated surface when immersed in drug solution. Only 1.4 \% from the sensitizer present in solution adsorbed onto the Teflon/PVAA surface, while 37.5 \% of the sensitizer adsorbed onto fluorinated PVG. Native Vycor show very poor repelling property with 76.3\% of the dye adsorbed onto its surface. This could be explained by the presence of a higher number of O-H bonds on the PVG surface which can serve as adsorption sites for the sensitizer. Adsorption was a time dependent process, for Teflon/PVA the adsorption of chlorin e\textsubscript{6}-trimethylester reached 0.8 \% after 30 min and 1.4 \% after 4h.

The new Teflon/PVA surface shows the most reduced sensitizer adsorptive affinity which makes the Teflon-life surface a promising alternative sensitizer support for drug delivery for our PDT device.

Adsorption of chlorin e\textsubscript{6}-trimethylester in 1-butanol onto native Vycor, Fluorinated Vycor and Teflon/PVA surfaces, were followed at \( \lambda = 664 \text{nm} \). The samples were immersed in 1-butanol (5mL), which contained chlorin e\textsubscript{6}-trimethylester (0.96x10\textsuperscript{-5}M). The samples were removed at the indicated times.
Table 10. Nanomoles and percent adsorption of chlorin e₆-trimethylester onto native Vycor, fluorinated Vycor and Teflon/PVA surfaces in 1-butanol.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Native Vycor nmoles (and %) adsorbed</th>
<th>Fluorinated Vycor (and %) adsorbed</th>
<th>Teflon/PVA (and %) adsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 nmoles (0%)</td>
<td>0 nmoles (0%)</td>
<td>0 nmoles (0%)</td>
</tr>
<tr>
<td>30</td>
<td>10.49 nmoles (21.9 %)</td>
<td>3.22 nmoles (6.7%)</td>
<td>0.16 nmoles (0.3%)</td>
</tr>
<tr>
<td>60</td>
<td>14.35 nmoles (29.9 %)</td>
<td>4.89 nmoles (10.2%)</td>
<td>0.32 nmoles (0.7%)</td>
</tr>
<tr>
<td>90</td>
<td>20.53 nmoles (42.8 %)</td>
<td>6.12 nmoles (12.8%)</td>
<td>0.32 nmoles (0.7%)</td>
</tr>
<tr>
<td>120</td>
<td>25.83 nmoles (53.8 %)</td>
<td>7.19 nmoles (15.0%)</td>
<td>0.48 nmoles (1.0%)</td>
</tr>
<tr>
<td>150</td>
<td>27.15 nmoles (56.6 %)</td>
<td>8.26 nmoles (17.2%)</td>
<td>0.48 nmoles (1.0%)</td>
</tr>
<tr>
<td>180</td>
<td>28.03 nmoles (58.4 %)</td>
<td>8.71 nmoles (18.1%)</td>
<td>0.48 nmoles (1.0%)</td>
</tr>
<tr>
<td>210</td>
<td>28.80 nmoles (60.0 %)</td>
<td>8.87 nmoles (18.5%)</td>
<td>0.63 nmoles (1.3%)</td>
</tr>
<tr>
<td>240</td>
<td>29.47 nmoles (61.4 %)</td>
<td>9.63 nmoles (20.1%)</td>
<td>0.63 nmoles (1.3%)</td>
</tr>
</tbody>
</table>

Sample pieces were immersed in 1-butanol solution containing chlorin e₆-trimethylester. The ability of the native Vycor, Fluorinated Vycor and Teflon/PVA surfaces to resist drug adsorption is summarized in table above. As previously observed both fluorinated surfaces, Teflon/PVA and fluorinated PVG, have a higher resistance to drug adsorption. The Teflon/PVA surface shows excellent repelling properties, with 1.3% dye adsorption in 4 h while 20.1% of dye adsorbed onto Fluorinated Vycor surface. Nonflorinated PVG surface has a very low resistance to sensitizer adsorption with 61.4% of dye present in solution being adsorbed onto its surface after 4h which could be explained by the presence of high number of O-H bonds on its surface (vs fluorinated surfaces) which can serve as adsorption sites.
In presence of internal light irradiation and oxygen purging from the PDT device, tri-PEG chlorin e₆ was successfully photoreleased from the Teflon/PVA probe tip in butanol. The amount of dye detected in solution after 75 min was ~18 nmoles (45.13%). The results collected so far prove that Teflon/PVA surface has the potential to be used as a platform for drug delivery for future application in the area of PDT for human cancer.

2.3 Conclusion. This work described two types of fluorinated surfaces, as supports for the photorelease of a PEGylated sensitizer drug and their ability to release the sensitizer drug was examined. The sensitzers, chlorin derivatives that were PEGylated with triethylene glycol, were bound to surface OH groups of (A) fluorinated silica (Vycor glass monolith coated with nonafluorosilane), and (B) a Teflon/polyvinyl alcohol nanocomposite

Synthetic, adsorption, sensitizer release and pO₂ measurements were described. It is useful investigating integrative polymer methods with new photoactive materials for fiber optics because it addresses current PDT limitations. The outcome was establishing which design and coating is best for the probe tip to achieve ¹O₂ and sensitizer delivered directionally and examined their ability to photorelease a PEGylated sensitizer drug. The presence of C−F bonds in the polymers was beneficial for high O₂ solubility, repelling action, and low physical quenching of ¹O₂.

The data also shows the ability of the new fluorinated surfaces to resist the adsorption of PEGylated sensitizer. The finding was that two fluorinated surfaces yield repellent characteristics to discharge the sensitizer but by different mechanisms. We show that these surfaces have (i) high solubility of ground-state oxygen, which is advantageous for photooxidation chemistry; (ii) relatively low adsorptivity for the PEGylated sensitizer, for good turnout; (iii) facile breakage of the ethene linker bond; and (iv) longer singlet oxygen lifetimes (τₐ) because the C−F bonds sap the polymer’s ability to physically quench ¹O₂ (i.e.,the paucity of C−H and O− H bonds limits unwanted vibrational relaxation of ¹O₂ to ³O₂).

Future directions: Designing photoactive repellent surfaces so that drugs are released rather than retained is largely an empirical endeavor and remains a challenge. However, opportunities exist by integrating methodologies from the photosciences to engineering to address the problem. We have successfully synthesized surfaces that are fairly repellent (i.e. Teflon-like) which photorelease a PEGylated sensitizer drug to surrounding solution, however further improvements could be made. The surfaces could be made into device tips to discharge controlled sensitizer
quantities locally, and will need to deliver at least 10 nmol of sensitizer to ensure a ~1.0 nmol/mL
value is reached tissue repair or PDT in vivo.

We have synthetic experience in producing fluorine-rich, hydrophobic materials, PEGylated
sensitizers, and with all techniques proposed our current prototype device could be used combined
with the new fluorinated materials in animal studies. We will turn to in vivo experiments and we
aim to display the surfaces’ utility in mouse tumor model experiments. Also, the fluorinated
surfaces provide intriguing possibilities for generating new types of device tips based on singlet
oxygen for tissue repair or photodynamic action.

2.4 References
4565.
Nowakowaska, M.; Vattulainen, I.; Kepczynski, M.; Rog, T. J. Phys. Chem. B. 2014, 118, 144-
151.
10 Klán, P.; Šolomek, T.; Bochet, C.G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.;


Chapter 3. Design of New Probe tips for the Microoptic Device: Teflon-like and Silica

Xerogel Probe Tips

3.1 Introduction. The current work shows efforts on developing new probe tips for our PDT device. A key step in our method is selecting the appropriate solid support for the sensitizer, so that the site specific delivery of sensitizer and singlet oxygen is maximized. The development of new biocompatible microoptic probe tips for PDT applications will focus on making microtips more drug repellent to minimize sensitizer readsorption (self-cleaning properties) and, by their virtues, to offer an increase in the ground-state oxygen solubility.

Studies have shown that PVG fluorination increases the probe tips self-repelling properties and cleavage efficiency by 15 %.\(^1\) Our aim is to include, as one component of our probe tip material, a highly hydrophobic constituent. We also aim to prepare a porous probe tip surface, which will allow oxygen to permeate through the probe tip, feature that is advantageous when treating hypoxic tumors. Two new types of probe tips are proposed for synthesis: a Teflon-like probe tip; and a silica monolith probe tip (synthesized via sol-gel).

**Teflon-like Probe Tip.** As an alternative to probe tip surface fluorination,\(^1\)-\(^3\) we propose to construct our microtip surface from Poly(tetrafluoroethylene), PTFE and Polyvinyl alcohol, (PVA). PTFE should increase the hydrophobicity of the tip and also, like fluorinated artificial bloods\(^4\) and ionic liquids\(^5\), should increase the ground-state oxygen solubility. The alcohol group of PVA will eventually be used for covalent surface attachment of the sensitizer. In addition, the unique characteristics of PTFE, such as outstanding thermal and chemical resistance, make it a suitable building material for our fiber tip surface. The Teflon/PVA blend ratio can be adjusted to reach the desired hydrophobic properties. PVA offers an additional advantage for surface functionalization over the commonly used PVG. While PVG requires treatment with large excess of nonafluorohexamethoxysilane making the degree of functionalization difficult to control, PVA offers a simpler more controlled functionalization method via SN\(_2\) substitution. Thus, utilizing a combination of PTFE and PVA for microtip construction gives the advantages of tunable tip hydrophobicity, as well as easier and more controlled functionalization. This polymer system should enable us to maximize the site-specific delivery of photosensitizer and singlet oxygen to tumor sites. Other advantages of using the Teflon/PVA microtip are easy of synthesis and low cost.
Our goals are to (1) synthesize stable Teflon-like surfaces of different blend ratios (Teflon: PVA 2:1, 4:1, 6:1); (2) characterize the new surface (3) determine the solvent and thermal stability of the Teflon-like microtip; (4) measure the oxygen permeability through the newly synthetized probe tip; and (5) evaluate drug repellent properties of the Teflon-like surface.

**Silica Monolith Probe Tip.** We also examined an alternative approach, where a porous TMOS/MeOH/H₂O xerogel was proposed to be used as an alternative probe tip surface for the sensitizer support. TMOS/MeOH/H₂O xerogels are porous silica matrices with tailorable porosity and surface area synthesized by well-known sol-gel techniques. For example, a base catalysis yields a transparent (50 %T at 367 nm vs. air), porous, “glass-like” material composed of interlocking silica nodules with the spaces between the nodules defining a random distribution of interconnected pores throughout the material. AFM imaging reveals the silica nodules are not integral units, but are composed of smaller silica nodules on the order of ≤1 nm. The surfaces of the nodules are relatively smooth, containing a relatively small number of 3-4 nm high and wide “stalagmite-like” features. Chemically, the nodule surfaces possess individual and associated, or hydrogen bonded, silanol, Si-OH, groups. The surface area of the xerogel is 508±18 m²/g, and N₂ adsorption-desorption isotherms yield pore diameters ranging from 0.5-2000 nm in diameter.

The object of this work is to investigate strategies for synthesizing crack-free porous silica monoliths surfaces using sol-gel method. New xerogel probe tips surfaces will be prepared at room temperature via sol-gel method, using the acid based catalyzed method. Our goals are to (1) synthesize stable silica monoliths using the sol-gel acid-based catalyzed method; (2) use drying control agents Dimethylformamide (DMF) to reduce risk of cracking during drying; (3) prepare sensitizer doped glass; (4) prepare sensitizer coated glass and (5) test singlet oxygen production at the xerogel surfaces.

**3.2 Results**

**3.2.1 Synthesis of Teflon-like Probe Tips for the Microoptic Device.** As mentioned above, my work focuses on the possibility of improving the efficiency of our current PDT device by preparing a new probe tip material with increase drug resistance and ground state oxygen solubility. A new probe tip material is proposed for synthesis, which will be made of Teflon and PVA. The new surface (Figure 19) was synthesized in an aqueous solution from a mixture of PTFE emulsion (aqueous based) and PVA (known to dissolve in water), using a modified
procedure previously reported by Huang et al. and Avela et al.\textsuperscript{8,9} Aqueous 5 % PVA solution was prepared first by dissolving PVA powder in distilled water at 90 °C for 5 hours under constant stirring. The solution was cooled to room temperature and a PTFE suspension with characteristics shown in Table 11 was added to the PVA solution in 2:1, 4:1, or 6:1 Teflon/PVA mass ratio.

Table 11. Characteristics of PTFE emulsion.

<table>
<thead>
<tr>
<th>Solid content (wt %)</th>
<th>Nonionic surfactant content (wt %)</th>
<th>Average particle size (nm)</th>
<th>Density (g/ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>5.9</td>
<td>22.3</td>
<td>2.20</td>
<td>10.8</td>
</tr>
</tbody>
</table>

The Teflon emulsion contains PTFE nanoparticles of 22.3 nm average size and TERGITOL\textsuperscript{TM} TMN-10, a nonionic surfactant which is known to have a narrow gel range.

\[
\begin{align*}
\text{(H}_2\text{C} & \text{CH)}_n + \text{(F}_2\text{C} & \text{C})_n \rightarrow \text{OH} \\
\end{align*}
\]

Figure 19. PVA and PTFE components included in the new surface material.

Figure 20. Chemical structure of the nonionic surfactant, TERGITOL\textsuperscript{TM} TMN-10 present in the PTFE emulsion.

The mixture was stirred at room temperature and atmospheric pressure for 2.5 hours using a mechanical agitator. Samples were placed in a mold and dried at room temperature and atmospheric pressure for one week. After drying, the new surface was crack free and probe tips of desired dimensions were shaped.

3.2.1.1 Characterization of Teflon-like Probes Surface. The chemical composition of all three blends of PVA-Teflon material (1:2, 1:4, 1:6) was investigated by FTIR. Two bands near 1203 and 1147 cm\textsuperscript{-1} were assigned to the C-F stretching vibrations of PTFE, and the two bands
around 2937 and 3315 were characteristic for PVA. The broad band observed from 3100-3600 cm$^{-1}$ was assigned to O-H stretching. The 2850-2950 cm$^{-1}$ band was assigned to C-H alkyl stretching. The peaks at 1080-1150 cm$^{-1}$ and 1419-1325 cm$^{-1}$ were attributed to stretching vibration of C=O and C-O of the acetate group which remained unhydrolized in the PVA polymer. It was confirmed in the IR spectra that, as the amount of Teflon content to the PVA increases, –OH peak intensity reduces. IR spectra of PVA-Teflon polymer with different fluorine content is shown below (Figure 21).

![FTIR for Teflon Polymer](image)

**Figure 21.** IR spectra of polymer having different Teflon contents have been shown Teflon/PVA 6:1 (green), Teflon/PVA 4:1 (blue) and Teflon/PVA 2:1 (red). IR spectral peak for –OH stretching frequency at 3296.77 cm$^{-1}$ decreases as the amount of PVA contents decreases in the polymer. Also an increase of peak intensity for the C-F stretching vibrations of PTFE is observed as the amount of PTFE is higher in the polymer.

Measurements of Teflon- PVA material were carried out using an FTIR spectrometer (Mattson, 6020-Galaxy Series) in transmittance mode in the frequency range of 4200–700 cm$^{-1}$.

Morphological characteristics of the material and the degree of PTFE nanoparticles dispersion within the polymeric matrix were investigated by scanning electron microscopy (SEM), see Figure 13. Analysis was done on the polymer which contained the higher amount of PTFE particles, Teflon/PVA 6:1. Samples were mounted on a specimen stubs and coated with a thin Gold/Palladium layer by the means of a sputter coating apparatus. The coated samples were
examined by SEM operating at 10kV. Surface morphology investigated by SEM at 1720X and 20000X magnification are shown in Figure 13.

The scanning electron microscope analysis shows that the new Teflon-like surface, consists of a smooth PVA phase with high content of submicron PTFE particles (22.3nm). PTFE nanoparticles were used without any coating agent and we observed that they were able to disperse themselves in the matrix generating a good interfacial adhesion. This can be explained by the organic polymeric nature of PTFE particles and its compatibility with the polymeric matrix. The SEM image of the Teflon/PVA surface also shown that there is no clustering among the nanoparticles in the PVA matrix.

3.2.1.2 Stability of Teflon/PVA Surface. Solvent compatibility studies were performed for the new Teflon/PVA surface. Solid polymers were soaked in common organic solvents (see table 12) for 24 hours and monitored for dissolution and other physical changes.

Table 12. Polymers stability in organic solvents at room temperature for 24 h.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Teflon/PVA 6:1</th>
<th>Teflon/PVA 4:1</th>
<th>Teflon/PVA 2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Swells 20% volume increase</td>
<td>Swells 40% volume increase</td>
<td>Swells 60% volume increase</td>
</tr>
<tr>
<td>Toluene</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Methanol</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>DMF</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Octanol</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Acetone</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>10% DMSO in water</td>
<td>Swells</td>
<td>Swells</td>
<td>Swells</td>
</tr>
<tr>
<td>100% DMSO</td>
<td>Not stable 50% disintegrated</td>
<td>Not stable 60% disintegrated</td>
<td>Not stable 70% disintegrated</td>
</tr>
</tbody>
</table>
No changes were observed when the Teflon surfaces were treated with acetone, toluene, dichloromethane, dimethylformamide (DMF), and octanol. Treatment with water caused the polymer surface to swell to approximately 20-60% of original volume after 24 h, depending on the amount of PVA present in the blend. The polymer with a higher content of Teflon (Teflon/PVE 6:1) showed the least amount of volume increase when soaked in water for 24h. We also observed that the swelling is linear with time for all three types of blends.

A 24 h treatment of the Teflon-like surfaces with DMSO causes partial dissolution of the polymers, see Figures 22 and 23. The surface with a higher content of Teflon (Teflon/PVE 6:1) showed the least amount of dissolution in DMSO after 24h. Presence of PTFE nanoparticles improves the resistance of PVA nanocomposites to degradation. If the Teflon surfaces are not removed from DMSO after 24 h and are allowed to remained immersed in DMSO for a total of 48 h, we observed that all three types of surfaces (Teflon/PVA 2:1, 4:1, and 6:1) completely disintegrated in the solvent, due to the strong solvation of PVA particles by DMSO.

**Figure 22.** Stability of Teflon polymers in 100% DMSO at room temperature after 24h: (left) Teflon/PVA 6:1, (middle) Teflon/PVA 4:1 and (right) Teflon/PVA 2:1.

**Figure 23.** Stability of Teflon/PVA in 100% DMSO with time 24h (left vial), 6h (middle vial), and initial time (right).

We concluded that the solvent stability of the Teflon/PVA composites material is strongly related to the amount of Teflon present in the polymer matrix. The polymer surface with the highest
content of Teflon (Teflon/PVE 6:1) showed the least amount of volume increase when immersed in water for 24h, and the smallest amount of dissolution in DMSO. For the Teflon/PVA 2:1 surface these trends were reversed.

The thermal stability of the Teflon/PVA surfaces was also tested to determine whether the new surface can withstand synthetic conditions for surface functionalization. As previously described, sensitizer attachment onto the PVG surface requires reflux in toluene at 100 °C for 48 hours. We want to investigate whether the Teflon surface has the ability to withstand similar reactions conditions.

Polymer samples with different Teflon blend ratios (Teflon/PVA 2:1, 4:1 and 6:1) were placed in a round bottom flask and refluxed under nitrogen for 48 h in toluene at 110 °C and then evaluated for any physical changes. As seen from Figure 24, the polymer with the highest Teflon ratio showed no thermal degradation while the other two polymers showed a very little color change (yellowing) which might be due to slight decomposition of PVA.

**Figure 24.** Polymer Teflon/PVA 2:1 (left), Teflon/PVA 4:1 (middle), Teflon/PVA 6:1 (right) after toluene reflux at 110 °C, showing a stable Teflon/PVA 6:1 surface and slight degraded Teflon/PVA surfaces with 4:1 and 2:1 ratios.

We also tested the thermal stability of the Teflon/PVA 6:1 blend by heating the samples in a conventional oven, under atmospheric pressure, from 50 to 450 °C at a rate of 10 °C/min. We observed that when the polymer was heated from 50 to 120 °C there were no physical changes.

**Figure 25.** Teflon/ PVA (6:1) surface after oven drying at 180 °C, showing PVA decomposition.
At temperatures above 130 °C we observed thermal instability as a slight color change. A strong yellowing color, associated with PVA decomposition, is observed at 180 °C (see Figure 25) which is assumed to be due to the decomposition of the PVA. After the sintering process the PVA has been decomposed completely and only a pure PTFE porous membrane is left.

3.2.1.3 Oxygen Permeability through the New Teflon-like Tip. Tumor cells are often “hypoxic”, which is problematic since ground-state triplet O$_2$ is required for photodynamic therapy. Flowing O$_2$ within the optical fiber to hypoxic tumors will enable greater $^1$O$_2$ uptake and enhanced photooxidative damage of the tumor. The fiber optic technique aims to control the concentration of O$_2$ in the medium. By achieving high local concentrations of sensitizer and O$_2$, concurrent with high excitation intensities, the new fiber optic approach will minimize damage to surrounding tissue. Ground-state O$_2$ is mildly toxic itself and saturating the environment beyond a tumor site is not desirable.

First we attempted to measure the O$_2$ flow through the PVG cap (bare and sensitizer coated) using our fiber optic device. The hollow fiber allowed for O$_2$ delivery into the surrounding media, because of the 40 Å pores$^{10}$ of the porous Vycor tip.

Table 13. Oxygen permeability through porous PVG fiber tip.

<table>
<thead>
<tr>
<th>Time</th>
<th>Bare PVG cap</th>
<th>Sensitizer coated PVG cap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p.p.m.$^a$</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>O$_2$ in H$_2$O</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>8.7</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>8.8</td>
<td>1.2</td>
</tr>
<tr>
<td>20</td>
<td>8.9</td>
<td>2.3</td>
</tr>
<tr>
<td>30</td>
<td>9.1</td>
<td>4.6</td>
</tr>
<tr>
<td>60</td>
<td>9.6</td>
<td>10.3</td>
</tr>
</tbody>
</table>

$^a$ Quantities of oxygen delivered through the hollow fiber optic cable (3 m) with metal tip into 7.0 mL of H$_2$O from a compressed oxygen tank at room temperature and 760 torr, with the gas regulator set at 10 PSI. PVG fiber tip dimensions: cylinder shape with a length of 8.0 mm, diameter of 5.0 mm and hole (3.0 mm diameter · 2.0 mm length).
Oxygen transmission through the probe tip into the H2O solution occurred at a rate of 0.16 p.p.m. min\(^{-1}\) at 10 PSI as measured by a dissolved oxygen meter (table 13). A ca 9 p.p.m. egress of oxygen gas was observed over a 1 h period, which indicates that a significant O\(_2\) pressure drop occurs at the fiber tip/solution boundary. It is tempting to speculate that the ca 1 % O\(_2\) purge rate increase in the bare vs sensitizer-coated tip provides purge assistance in the release of the sensitizer (albeit marginal in the current device configuration), since a previous study used a gas-purge method to facilitate the desorption of 1,2-dibromoethane from microporous soils.\(^{11}\)

Changes in oxygen concentration (measured at certain intervals for 1h) and oxygen pressure (continuously monitored for the course of 15 minutes) were also measured in an aqueous solution when oxygen is delivered through the Teflon/PVA or PVG cap in solution.

An oxygen meter oxylite was used to measure oxygen transmission through the porous Teflon/PVA tip into 7 mL water over the course of an hour. Results are summarized in table 14 below. A 22.6 % increase of O\(_2\) concentration is observed when oxygen is delivered through the Teflon/PVA tip after 1 h (Table 14) relative to previously reported 10.3 % when O\(_2\) is delivered through the PVG cap.\(^{12}\)

**Table 14.** Quantities of oxygen delivered through Teflon-PVA cap attached to fiber optic cable.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>ppm of O(_2) in water</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>8.6</td>
<td>2.4</td>
</tr>
<tr>
<td>20</td>
<td>8.9</td>
<td>5.0</td>
</tr>
<tr>
<td>30</td>
<td>9.4</td>
<td>11.9</td>
</tr>
<tr>
<td>60</td>
<td>10.3</td>
<td>22.6</td>
</tr>
</tbody>
</table>

Oxygen was delivered through the hollow fiber optic cable via a metal tip into 7 mL of H\(_2\)O from an oxygen tank at 10 PSI, at room temperature and 760 torr. Teflon/PVA fiber dimensions: cylindrical shape with length of 8.0 mm, diameter of 5.00 mm and a hole (3 mm diameter x 2.0 mm length).
Using a very sensitive pO₂ sensor, an OxyLite Pro with real time recording, we continuously monitored changes in oxygen pressure in an aqueous solution when oxygen was delivered through the porous PVG and Teflon/PVA cap in 1 mL of water. The pO₂ was continuously monitored for 15 minutes and results are summarized table 15 below.

**Table 15.** Changes in oxygen pressure and pO₂ levels when O₂ is delivered through the Teflon/PVA or PVG cap in 1 mL of water.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Teflon/PVA cap</th>
<th>PVG cap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pO₂ mmHg</td>
<td>nmoles</td>
</tr>
<tr>
<td>0</td>
<td>19.5</td>
<td>33</td>
</tr>
<tr>
<td>1</td>
<td>35.1</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>52.8</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>63.1</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>74.4</td>
<td>127</td>
</tr>
<tr>
<td>5</td>
<td>79.9</td>
<td>137</td>
</tr>
<tr>
<td>6</td>
<td>87.4</td>
<td>150</td>
</tr>
<tr>
<td>7</td>
<td>94.1</td>
<td>161</td>
</tr>
<tr>
<td>8</td>
<td>99.6</td>
<td>170</td>
</tr>
<tr>
<td>9</td>
<td>103.9</td>
<td>178</td>
</tr>
<tr>
<td>10</td>
<td>107.6</td>
<td>184</td>
</tr>
<tr>
<td>11</td>
<td>113.3</td>
<td>190</td>
</tr>
<tr>
<td>12</td>
<td>115.3</td>
<td>197</td>
</tr>
<tr>
<td>13</td>
<td>118.3</td>
<td>202</td>
</tr>
<tr>
<td>14</td>
<td>121</td>
<td>207</td>
</tr>
<tr>
<td>15</td>
<td>123.3</td>
<td>211</td>
</tr>
</tbody>
</table>

Oxygen was delivered through the hollow fiber optic cable via a metal tip into 1 mL of H₂O from an oxygen tank at 7 PSI, at room temperature and 760 torr. Teflon/PVA fiber dimensions:
cylindrical shape with length of 8.0 mm, diameter of 5.00 mm and a hole (3 mm diameter x 2.0 mm length).

Figure 26. Time course of pO$_2$ pressure in 1mL of water when Oxygen is delivered through Teflon/PVA (black) or PVG (red) cap into the aqueous solution at 7 psi.

We observed that when O$_2$ was delivered through the Teflon/PVA cap for 15 minutes in the aqueous media, the pO$_2$ increases 103 mmHg, from 19.5 mmHg at t=0 to 123.3 mmHg at t=15 min (see Figure 26). This constitutes a 535% increase in oxygen pressure (see Figure 27). When oxygen is delivered through the PVG tip for 15 minutes the pO$_2$ increases 62 mmHg from 22.8 mmHg at t=0 to 84.8 mmHg at t=15 min which is a 272% increase in O$_2$ pressure.

The above collected data shows that oxygen permeability through the Teflon/PVA probe tip is superior when compared with a PVG tip. Both oxygen concentration and oxygen pressure are higher in an aqueous solution when oxygen is delivered through the fiber containing the Teflon/PVA probe tip.
Figure 27. Percent increase of oxygen pressure with time when O₂ is delivered through the Teflon/PVA or PVG cap in 1 mL of water. Teflon/PVA (black) and PVG (red) cap.

3.2.1.4 Evaluation of Self-repelling Properties of the New Teflon-based Microtip. As shown previously, the release of dye into the aqueous media following photocleavage is hampered by dye readsorption onto the PVG tip surface.¹² Modifying the PVG surface with nonafluoro hexane trimethoxy silane induces a partially inert surface and thus minimizes dye readsorption.¹³,¹⁴ This fluorinated fiber optic tip, attached to the photolabile spacer alkene-sensitizer, reduced the sensitizer adsorption onto the tip surface after the alkene bond was cleaved. We found that dye readsorption for the fluorinated probe tip drops to 9% as compared to 90% in non-fluorinated PVG, contributing to an overall photocleavage efficiency increase of 15%.¹³ As mentioned earlier, the PTFE content of the new Teflon/PVA tip should have a similar effect on dye-readsorption to PVG fluorination. Increasing PTFE content is hypothesized to maximize the self-cleaning properties of the tip surface, thus maximizing sensitizer release.

The ability of the new Teflon/PVA surface to resist dye adsorption was evaluated for all 3 types of polymer Teflon/PVA (6:1, 4:1, 2:1) using a variety of dyes with different hydrophobicity. The adsorptive affinity was evaluated by soaking solid polymers in a dye containing solution, and monitoring the decrease of dye concentration in solution by UV. Results are summarized in table 16 below.
Table 16. Nanomoles and percent adsorption of dyes adsorbed onto Teflon/PVA (6:1, 4:1, 2:1) surfaces in different solvents after 3h. Loadings onto the Teflon/PVA ranged from 0.8 nmol TPP onto Teflon/PVA 6:1 to 139 nmoles of meso-Tetra (N-methyl 4-piridyl) porphine tetratosylate adsorbed onto Teflon/PVA 2:1.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Mole of dye adsorbed per 1g of Teflon/PVA 2:1</th>
<th>Mole of dye adsorbed per 1g Teflon/PVA 4:1</th>
<th>Mole of dye adsorbed per 1g of Teflon/PVA 6:1</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose Bengal</td>
<td>129 nmoles (67.78%)</td>
<td>69.2 nmoles (36.4 %)</td>
<td>38.5 nmoles (20.3 %)</td>
<td>H₂O</td>
</tr>
<tr>
<td>Phthalocyanine tetrascousionic acid</td>
<td>88.9 nmoles (46.78%)</td>
<td>47.8 nmoles (25.2%)</td>
<td>26.2 nmoles (13.8%)</td>
<td>H₂O</td>
</tr>
<tr>
<td>meso-Tetra porphine tetratosylate</td>
<td>139 nmoles (73.10%)</td>
<td>121 nmoles (63.5%)</td>
<td>101 nmoles (53.4%)</td>
<td>H₂O</td>
</tr>
<tr>
<td>Rhodine G7</td>
<td>57.4 nmoles (30.21%)</td>
<td>41.6 nmoles (21.9%)</td>
<td>33.6 nmoles (17.7%)</td>
<td>H₂O</td>
</tr>
<tr>
<td>TPP (mesotetra phenyl porphine)</td>
<td>2.9 nmoles (1.52%)</td>
<td>1.6 nmoles (0.8%)</td>
<td>0.8 nmoles (0.4%)</td>
<td>100% toluene</td>
</tr>
<tr>
<td>Pheophorbide a</td>
<td>11.8 nmoles (6.05%)</td>
<td>7.3 nmoles (3.7%)</td>
<td>3.8 nmoles (2.0%)</td>
<td>10% DMSO in H₂O</td>
</tr>
<tr>
<td>Chlorin e6</td>
<td>37.6 nmoles (19.8%)</td>
<td>17.8 nmoles (9.1%)</td>
<td>9.9 nmoles (5.2%)</td>
<td>10% DMSO in H₂O</td>
</tr>
</tbody>
</table>

As seen in Table 16 and Figure 28, adsorption to the Teflon/PVA surfaces ranges from 0.8 nmoles of TPP adsorbed to the Teflon/PVA 6:1 to 139 nmoles of Meso tetra porphine tetratosylate adsorbed to the Teflon/PVA 2:1, depending on the type of dye or polymer blend. We observed that the resistance to drug adsorption increases as the amount of PTFE present in the blend increases (see Figure 28). The surface containing 6:1 Teflon/PVA showed to have the highest repellent properties due to higher amount of fluorine atoms in the blend, which add inertnes to the surface, increasing its self cleaning properties.
Figure 28. Image showing repellent properties of Teflon/PVA (6:1 first row, 4:1 middle row, 2:1 bottom row) surfaces to various dyes that were sitting in a 2.0 mL solution containing (10 mM dye solutions: rose bengal, phthalocyanine tetrasyonic acid, meso tetra porphine tetratosylate, rhodine G7, TPP (mesotetra phenyl porphine), pheophorbide a and chlorin e₆ after 3h.

![Image showing repellent properties of Teflon/PVA surfaces to various dyes](image)

Table 17. Percent adsorption of rhodin G7 onto native PVG, fluorinated PVG and Teflon/PVA surface. The Teflon/PVA samples were immersed in water (10mL) which contained rhodin G7 (10⁻⁵ M). The samples were removed from the solution after 4 h.

<table>
<thead>
<tr>
<th>Surface</th>
<th>PVG</th>
<th>Fluorinated PVG</th>
<th>Teflon/PVA 6:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Rhodine G7 adsorbed</td>
<td>75.5</td>
<td>31.2</td>
<td>16.9</td>
</tr>
<tr>
<td>Number of C-F bonds</td>
<td>0</td>
<td>7.8x10²¹</td>
<td>9.6x10²¹</td>
</tr>
</tbody>
</table>

We also compared adsorptive affinities of Teflon/PVA surfaces with the currently used PVG surface. Rhodin G7 was chosen as the sample dye and we monitored its adsorption onto the native PVG, fluorinated PVG, and new Teflon/PVA 6:1 composite material. All PVG and the new Teflon/PVA samples were soaked in 10 mL of 10⁻⁵ M dye solution for 4 h. The adsorption of sensitizer onto the probe tip was checked by UV-vis spectrophotometry by monitoring the decrease in dye concentration.

We observed that fluorinated surfaces (fluorinated PVG and Teflon/PVA) show a higher drug resistance when compared to plain PVG. 75.5 % of rhodin G7 present in the solution adsorbed to the PVG surface, while only 31.2 % and 16.9 % adsorbed to fluorinated PVG and Teflon/PVA (see table 17). When self-cleaning properties of the fluorinated surfaces were compared we observed that the surface with the highest resistance to rhodin G7 adsorption was Teflon/PVA 6:1.
For a better understanding of the results, we determined the amount of C-F bonds present on both fluorinated surfaces. The calculated number of C-F bonds on the Teflon/PVA surface was 9.6x10^{21}, while the number of C-F bonds present on the fluorinated PVG surface was 7.8x10^{21}. A higher number of C-F bonds present on the Teflon/PVA adds repellency to the surface increasing the new polymer resistance to rhodin G7 adsorption.

The work described above shows that Teflon/PVA surface has the ability to resist drug adsorption and makes it suitable to be used as an alternative probe tip material for sensitizer support for the fiber optic device.

**3.2.2 Preparation of Porous Silica Probe Tips via Sol-Gel.** Sol-gel materials cover a wide range of inorganic and inorganic/organic composite materials which they all share a similar preparation method. The sol-gel process involves generation of colloidal suspensions (“sols”) which are consequently converted to viscous gels and then to solid materials. The synthesis of materials by the sol–gel process generally involves the use of metal alkoxides, which undergo hydrolysis and condensation polymerization.

The sol–gel process can usually be divided into the following steps: hydrolysis, condensation, gelation, aging, drying, and densification.

**Hydrolysis:** \( \text{Si(OR)}_4 + \text{nH}_2\text{O} \rightarrow \text{Si(OR)}_{4-n}(\text{OH})_n + \text{nROH} \)

**Condensation:** \( \text{X}_3\text{SiOH} + \text{HOSiX'}_3 \rightarrow \text{X}_3\text{Si-O-SiX'}_3 + \text{H}_2\text{O} \) or

\( \text{X}_3\text{SiOR} + \text{HOSiX'}_3 \rightarrow \text{X}_3\text{Si-O-SiX'}_3 + \text{ROH} \)

**Gelation:** A “spanning cluster” across the vessel is formed, giving a network which entraps the remaining solution, with high viscosity.

**Aging:** Further cross-links are formed associated with gel shrinkage as covalent links replace non-bonded contacts and also there is a structural development associated with changes in pore size and pore wall strength.

**Drying:** Water, alcohol and other volatile components are loss.

**Densification:** Upon thermal treatment the open structure collapses and a dense structure is formed.

In the preparation of a silica glass, an appropriate alkoxide [e.g., tetraethoxysilane (TEOS),\text{Si(OC2H5)}_4, or tetramethoxysilicate (TMOS), \text{Si(OCH3)}_4] is mixed with water and a mutual solvent, such as ethanol or methanol, forms a solution. Hydrolysis leads to the formation
of silanol groups, Si–OH. These species are intermediates because they additionally react to form Si–O–Si groups. As the hydrolysis and condensation polymerization reactions continue, viscosity increases until the solution ceases to flow. Sol–gel transition is irreversible, and at this stage, the one-phase liquid is transformed to a two-phase system. The gel contains amorphous primary particles of variable size (5–10 nm or smaller) with an interstitial liquid phase. In this phase, the pores have yet to shrink and the liquid phase fills the pores. After gelation, gels are generally subjected to an aging treatment. Condensation reactions continue, increasing the degree of cross-linking in the network. The aging process is followed by drying, which involves the removal of the liquid phase and the formation of a xerogel. Ambient temperature evaporation is frequently employed, and there is considerable weight loss and shrinkage.

3.2.2.1 Synthesis of Silica Monoliths via Sol-Gel Acid-Based Catalyzed Method. Xerogel samples were prepared using Si(OC₂H₅)₄, H₂O, ethanol, HCl and NH₄OH with a total volume ratio of 2 mL: 8 mL: 8 mL: 2.5 µL: 10.8 µL and synthesized by a two-step acid base catalyzing method. This involves an initial acid reflux hydrolysis step followed by a base catalyst-induced polymerization.

First TEOS was hydrolyzed under reflux and acidic conditions in a solution with volume ratio TEOS: EtOH: H₂O:HCl equal to 2 mL: 8 mL: 2 mL:2.5 µL. Ethanol and TEOS were mixed in a beaker with a magnetic stirrer on a hot plate, and then the water (mixed with HCl) was added drop wise into the reaction vessel. The solution was heated and stirred for 1.5 h at 65 °C.

Second, the silanol groups were condensated under basic conditions. After 1.5h the remaining water and NH₄OH (6ml and 10.8 µL) were added to the above hydrolyzed TEOS solution and stirred for 1 minute. The solution was poured into 10x45mm disposable PS cuvettes. A gel formed in 30 minutes and the solutions were capped and aged for 2 weeks. After aging, the samples were uncapped and then cover with parafilm which had several holes poked in it to assure a very slow drying rate (to avoid cracking). Unfortunately 40% of the gels cracked during room temperature drying process when water, alcohol and other volatile components were loss. The remaining crack free gels were dried at room temperature for 2 weeks and the oven dried at 60 °C for 1 day, 130 °C 3 days, and 700 °C for 6 h. During thermal treatment the open structure collapsed and a denser structure is formed. The resulting xerogel samples look transparent and porous. Most monoliths were stable when shaped or cut using an Isomet low speed cutter, but very few had the mechanical
strength to withstand drilling, which is necessary when the silica monolith surface is to be coupled with the fiber.

**3.2.2.2 Increasing Silica Monoliths Strength: Drying Control Chemical Agents.** One problem we had in xerogel preparation was cracking during the drying step\textsuperscript{18,19}. In order to overcome this problem we are investigating the possibility of adding drying control chemical additives (DCCA’s) during sol-gel preparation. N,N-Dimethylformamide (DMF) is proposed to be used as a drying control chemical additive. One of the effects of DMF will have is to suppress occurrence of cracks and fractures in the process of drying wet gels.

A two-step acid-base catalyzed method was used for xerogel preparation: 7.5mL DMF, 10mL TEOS and 40mL EtOH were mixed using a stirring bar on a hot plate and 10mL of water mixed with 12.5μL HCl were added drop wise to the reaction vessel. The solution was stirred for 1.5 h at 65 °C. 17.5mL of water containing 57 μL NH\textsubscript{4}OH was added to the reaction mixture and stirred for 2 minutes. A gel formed in 30 minutes and the solution was capped and aged for 1 day. After aging the samples were dried at 60 °C for 1 day, 130 °C 3 days and 700 °C for 7 h. The resulting xerogel samples seemed to have a higher transparency than the xerogels obtained using previous procedure.

Besides the silica monoliths prepared with the aid of a DCCA, DMF, we also prepared a control sample, without the addition of any drying control agent DMF. When structures of the silica gel monolith prepared from tetramethoxysilane (TEOS) solutions with or without DMF were examined, we observed that dry gels prepared from the solutions containing DMF appeared to be crack free, transparent in color, and by visual assessment showed larger pores than dry gels prepared without DMF. When gels prepared from sol-gel method without DMF were assessed we observed that half of the dry gels, appeared to be cracked, transparent, and, by visual assessment, showed small pores.

We conclude that DCCA seems to be suppressing the occurrence of cracks and fractures in the process of drying wet gels. We decided to test DMF’s role in the drying process by exposing the drying gels to water or DMF which have very different surface tensions. We observed that when the drying gels prepared from solutions containing DMF were exposed to water which has a high-surface tension, cracks formed; but no cracks were observed when the drying gels were soaked in DMF. This was understood to show that the low surface tension of DMF may be one of the effects
in protecting the drying gel from crack generation. It was presumed that DMF vaporizes at last during the drying process and weakens the capillary force exerting on the silica network structure.

The use of DCCA significantly reduces the number of cracked gels during the drying process from 40% to less than 3%, but work still needs to be done in increasing their mechanical strength for drilling, which still remains a problem.

3.2.2.3 Synthesis of Dye Doped Glass. A main advantage that silica monoliths glass has its chemical, photochemical stability and very good optical properties. These properties can be used for preparation of dye doped glasses for optical application. One other benefit of dye entrapment in sol gel matrices is that it can offer dye protection from chemical degradation. Meso-tetra(N-methyl-4-pyridyl)porphine (see Figure 29) was introduced in glass by sol-gel method from TEOS with the aim to determine whether upon photochemical reaction singlet oxygen can be generated at the glass surface.

![Figure 29. Structure of meso-Tetra(N-methyl-4-pyridyl)porphine tetratosylate.](image)

Three batches of xerogel samples doped with a cationic dye were prepared by using the experimental method described in section above (in 3.2.2.2 section) with the addition of 1.5 ml of $10^{-3}$ M dye meso-tetra(N-methyl-4-pyridyl) porphine tetratosylate into the reaction vessel during the hydrolysis step. The cuvettes containing the samples were capped and sealed with paraffin films and kept at 45°C in a conventional oven for gelation. It was noticed that the gelation time varied from 3-10 minutes. After aging, the gels were dried at atmospheric pressure and room temperature for 10 days. The cuvettes were covered with parafilm with several needle holes to allow a slow evaporation. After aging and air-drying, the silica gels solidified and were easily
removed from the cuvette. On batch was dried at 100 °C for 5 days while the other batch were dried at ambient pressure in the temperature range 100-600 °C within 5 days. The samples were first dried at 100 °C (rate of 1 °C min⁻¹) for 1 days. Then the dried gels were then heated at different temperature (300, 400, 500, 600 °C) for 1h at a heating rate 100 °C h⁻¹.

This drying procedure has been found to be the key to obtaining crack-free monolithic xerogels. It was also notice also that the xerogels dried at all of the above temperature (300-600 °C) were crack free when placed in water. Mechanical properties were tested also by drilling 1 mm holes in the xerogel samples but only few xerogel samples (dried at 400 °C) were crack free.

The dye doped xerogel samples dried at a temperature of 300 °C still contained the dye in their structure. A slightly color fading of the xerogel was noticed upon drying at 400 °C. Above 500 °C the sensitizer decompose from the xerogel sample, all samples were white clear and transparent.

The dye doped glasses dried at 100 °C were used for the photooxidation experiment, because they retain the dye in their structure without any dye decomposition. The amount of dye doped in the sol-gel glass was determined and calculated to be ~500 nmol/g. This was determined by dividing the total number of moles added to the reaction mixture during hydrolysis (1.5 μmoles) to the total grams of dried xerogel obtained after synthesis (3 g).

Table 18. Mole of meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate doped in 0.1g xerogel.

<table>
<thead>
<tr>
<th>Amount Xerogel</th>
<th>Mole of dye doped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Trial 2</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Trial 3</td>
<td>0.1 g</td>
</tr>
</tbody>
</table>

About 0.1 g of xerogel doped with ~ 50 nmoles of dye was used for the photochemical reaction and tested whether it can generate singlet oxygen at the surface. [4+2]-cycloaddition reactions are often common in verifying the presence of singlet oxygen in solutions. In this work we used 9,10-anthracene dipropionate dianion as trapping agent to detect singlet oxygen generation from the dye-sol-gel surface. The photooxidation product is 9,10-anthracene-9,10-endo-peroxide dipropionate dianion, (Fig below) which was formed by [4+2]-cycloaddition reactions and is known to be the only product in the reaction of singlet oxygen with anthracene.
Dye doped glass previously loaded with ~50 nmoles was placed in a aqueous solution containing the antracene trap (5x10^{-5} M) and the reaction vessel was immersed in the Rayonet reactor and irradiated for 6 minutes. External ground-state oxygen was also supplied to the reaction mixture from an oxygen tank. Singlet oxygen generation from the sensitizer adsorbed sol-gel surface was tested every 30 s. The concentration of anthracene in solution was measured by monitoring the decrease of anthracene absorption at 378 nm by UV (see Figure 31). Percent decrease of anthracene with time is shown in Table 19 and Figure 32.

**Figure 30.** Photooxidation of anthracene dipropionate dianion at the sol-gel surface.

**Figure 31.** Time course of anthracene disappearance in solution during photolysis, monitored by UV at 378nm.
Table 19. Absorbance of 5x10^{-5} M anthracene solution at 30 seconds intervals.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Absorbance</th>
<th>% Anthracene disappeared</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.262</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0.194</td>
<td>10.6</td>
</tr>
<tr>
<td>90</td>
<td>0.179</td>
<td>22.1</td>
</tr>
<tr>
<td>120</td>
<td>0.165</td>
<td>31.2</td>
</tr>
<tr>
<td>150</td>
<td>0.151</td>
<td>41.8</td>
</tr>
<tr>
<td>180</td>
<td>0.129</td>
<td>52.1</td>
</tr>
<tr>
<td>210</td>
<td>0.119</td>
<td>63.1</td>
</tr>
<tr>
<td>240</td>
<td>0.106</td>
<td>72.3</td>
</tr>
<tr>
<td>270</td>
<td>0.090</td>
<td>81.4</td>
</tr>
<tr>
<td>300</td>
<td>0.067</td>
<td>90.1</td>
</tr>
<tr>
<td>330</td>
<td>0.059</td>
<td>99.2</td>
</tr>
</tbody>
</table>

We observed that the amount of anthracene present in solution decreased with time which confirms that singlet oxygen was generated at the device tip and successfully photooxidized our anthracene trap. Table 15 and Figure 27 show that 99.2% of antracene was photooxidized by the device tip in 330 s.
Figure 32. Percent anthracene dipropionate dianion disappearance with time.

We successfully observed that our dye doped surface, upon Rayonet light irradiation and oxygen purging, was able to generate singlet oxygen. Singlet oxygen was detected by its reaction with 9,10-anthracene dipropionate dianion, which disappeared from the solution in less than 5 minutes. The results show that the new silica monolith surface may be considered as an alternative support for the photooxidation reaction and has the ability to be integrated as a probe tip with the fiber optic device.

3.2.2.4 Preparation of Sol-Gel Hybrid Probe Tips with Adsorbed Cationic Porphyrine and Detection of Singlet Oxygen at the Silica Surface. We wanted to take advantage of the cation binding property of xerogel and we attempted to adsorb meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate onto its surface. The dye adsorbed xerogel was prepared by soaking for 24 h under stirring approximate 0.1 g of xerogel samples into 1.5 ml aqueous solution of 1x10^{-5} M, 1.7x10^{-5} M, 3.3x10^{-5} M and 5x10^{-5} M dye. The amount (moles) of sensitizer adsorbed onto xerogel was calculated from the absorbance (λ max 422 nm) difference of the solution before the xerogel was placed into the solution and the absorbance of same solution after the xerogel was removed. The colorless xerogel was converted to a deep red upon absorption in 24 h. The porphyrin adsorbed to the glass gave a stable material, which did not dissociate in water at room temperature. The amount of dye loaded onto 0.1 g xerogel is shown in the table 20 below.
Table 20. Mole of meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate loaded on approximate 0.1 g xerogel.

<table>
<thead>
<tr>
<th>g Xerogel</th>
<th>Molarity of Dye solution</th>
<th>Mole of dye initial</th>
<th>Mole of dye final (after 24h)</th>
<th>Moles of dye loaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.127</td>
<td>1x10^{-5}</td>
<td>1.5x10^{-8}</td>
<td>6.58x10^{-10}</td>
<td>1.4x10^{-8}</td>
</tr>
<tr>
<td>0.123</td>
<td>1.7x10^{-5}</td>
<td>4.25x10^{-8}</td>
<td>1.09x10^{-9}</td>
<td>4.1x10^{-8}</td>
</tr>
<tr>
<td>0.103</td>
<td>3.3x10^{-5}</td>
<td>1.65x10^{-7}</td>
<td>2.97x10^{-8}</td>
<td>1.35x10^{-7}</td>
</tr>
<tr>
<td>0.130</td>
<td>5x10^{-5}</td>
<td>3.75x10^{-7}</td>
<td>7.5x10^{-8}</td>
<td>3x10^{-7}</td>
</tr>
</tbody>
</table>

Singlet oxygen detection at the sensitizer coated sol-gel monolith surface was achieved by photooxidation of trans-2-methyl-2-pentenoate anion. This is an indirect analysis of singlet oxygen generation. Trans-2-methyl-2-pentenoate anion is photooxidized into 3-hydroperoxy-2-methylene pentanoic acid (Figure 33).

![Figure 33. Photooxidation of trans-2-methyl-2-pentenoate anion.](image)

0.1 g xerogel with adsorbed cationic dye was placed in a solution containing 11.6 μL trans-2-methyl-2-pentenoic acid and 0.0012g NaOH in 10 mL D₂O. Oxygen was bubbled into the solution for 10 minutes. Using a Rayonet reactor, photooxidation was carried out for 4h at room temperature. We found that singlet oxygen was generated in the aqueous solution upon irradiation of sensitizer coated xerogel surface.
Table 21. Percent yield of alkene oxidation after 4 h.

<table>
<thead>
<tr>
<th>g Xerogel</th>
<th>Mole of dye loaded</th>
<th>Percent Yield After 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.127</td>
<td>1.4x10^-8</td>
<td>47 %</td>
</tr>
<tr>
<td>0.123</td>
<td>4.1x10^-8</td>
<td>40 %</td>
</tr>
<tr>
<td>0.103</td>
<td>1.35x10^-7</td>
<td>43.9 %</td>
</tr>
<tr>
<td>0.130</td>
<td>3x10^-7</td>
<td>63 %</td>
</tr>
</tbody>
</table>

Aliquots from the reaction mixture were analyzed by \(^1\)H NMR in D\(_2\)O and percent yield was calculated and summarized in Table 21. The integrated methyl protons were compared with the methylene protons of adipic acid which was used as an internal standard. \(^1\)H NMR (D\(_2\)O) \(\delta\) \(^1\)H NMR (D\(_2\)O) 0.89 (t, \(J = 7.5\) Hz, 3H), 1.64 (m, 2H), 4.73 (t, \(J = 6.5\) Hz,1H), 5.54 (s, 1H), 5.92 (s, 1H). Hydroperoxy-2-methylene pentanoic acid was detected as the single product.

3.3 Conclusion. Two different types of probe tips for our PDT device were synthesized and their efficiency for use as sensitizer support was tested. The surfaces were Teflon/PVA nanocomposite and silica monolith. First, three types of teflon-like surfaces were synthesized (Teflon/PVA 2:1, 4:1, 6:1) and their thermal and solvent stability and drug repellent properties were compared. Resistance to drug adsorption was tested by soaking the surfaces in five different types of dyes and then monitoring by UV the amount dye in solution before and after analysis.

Results reveal that the Teflon/PVA 6:1 surface shows the highest drug repellency. When thermal and solvent stability was evaluated we observed that all Teflon/PVA surface were stable in most solvents. No changes were observed when all three blends of polymers were treated for 24 h with acetone, toluene, dichloromethane, DMF and octanol. Water caused the 6:1 polymer to swell to approximately 20 % of its original volume after 24h, while the Teflon/PVA 2:1 showed even a higher percentage of swelling ~ 60 %. DMSO causes partial dissolution of the 6:1 blend polymer in 24 h, while in 48h all three types of blends disintegrated completely. The surface containing the highest PTFE ratio, Teflon/PVA 6:1, was chosen for further work, due to its
superior thermal and solvent stability and also its superior self-cleaning properties, feature advantageous in drug release.

IR and SEM were used to analyze the polymer surface. Chemical composition of the PTFE: PVA 6:1, was confirmed by IR analysis, which reveals two bands near 1203 and 1147 cm\(^{-1}\), assigned to the C-F stretching vibrations of PTFE, and two bands around 2937 and 3315 cm\(^{-1}\), characteristic for PVA. SEM analysis reveals that the Teflon/PVA probe tip consists of a smooth PVA phase with high content of submicron PTFE particles of 22.3 nm.

An alternative sensitizer support made of silica monoliths was also prepared at room temperature using an acid-based catalyzed sol-gel method, and its mechanical strength was increased by using DCCA (drying control chemical agents). Two types of sensitizer-monolith support were synthesized: sensitizer coated probe tips and sensitizer doped probe tips. The ability of the new silica monolith surfaces to generated singlet oxygen was tested, and singlet oxygen was detected in water by chemical trapping with alkene, trans-2-methyl-2-pentenoic acid and anthracene derivative to give hydroperoxide or endoperoxide. Based on this study, the new sensitizer coated and doped glass monolith can be used as an alternative probe tip for the microoptic device.

An important feature of the two surfaces when integrating with our PDT device is to withstand the drilling process when microtips are coupled with the fiber optic device. When mechanical strength of the silica monolith was compared to Teflon/PVA surface, we observed that, under drilling conditions, silica monolith showed low mechanical strength (cracking), while Teflon/PVA was crack free. For these reasons we decided to continue our further work with Teflon/PVA surface.

3.4 References


17. Wright, J. D.; Sommerdijk. N.A.J.M. *Sol-Gel Materials: Chemistry and Applications*, **2001**


Chapter 4. Functionalization of Teflon/PVA Microptic Tip Surface Using Model Compounds and Chlorophyll Derivatives.

4.1 Introduction. Sensitizer immobilization onto the probe tip surface is a significant step in our method. Our aim is to synthesize hybrid Teflon-like microtips by covalent attachment of chlorophyll derivatives via a stable linkage to the Teflon/PVA polymer. O-Vinyl ethers have been advantageous for photocleaving reactions at the double bond. A number of them react with $^1$O$_2$ by a mechanism involving dioxetane intermediates.$^{1-4}$ We have previously developed a synthetic route for the covalent attachment of the hydrophobic sensitizer- pyropheophorbide-$a$ formate ester onto PVG polymers surface$^5$ which relies on trimethoxy silane chemistry. An advantage of the new Teflon/PVA surface has over the previously used PVG surface is that the alcohol groups of PVA in the Teflon/PVA polymers allow for direct covalent attachment of the alkyl chains. We propose to use alkylation reactions (Figure 34) to first attach to the Teflon/PVA surface 9-bromomethyl anthracene, as model compound, and then two sensitizer drugs, chlorin $e_6$ and pheophorbide-$a$.

![Figure 34. Bioconjugation of chlorophyll derivatives to Teflon/PVA surface via nucleophilic substitution.](image)

Such a route to functionalization has already been shown effective by alkylation of PVA hydrogel surfaces coated on polyethylene in the presence of a deprotonating agent (sodium ethoxide or potassium tertbutoxide), as described by Duncan et al.$^6$ Alkylation scheme of PVA hydrogel using alkyl halides is shown in Figure 35.
The first approach used for Teflon/PVA surface covalent attachment will use a similar alkylation reaction, as mentioned above in Figure 35, except that in our case sodium hydride, NaH, will be used as the deprotonating agent. For synthesis the hydride ions will deprotonate the surface hydroxyl groups of PVA from the Teflon/PVA surface, rendering them more nucleophillic, and the alkyl bromide will complete the attachment by the nucleophillic substitution of the deprotonated hydroxyl.

We are also interested in exploring a second strategy for sensitizer surface immobilization, and we plan to achieve this by first modifying the Teflon/PVA surface and then attach the organic compound to the modified Teflon/PVA surface. 9-hydroxymethyl anthracene will be used as model compound to test the proposed synthetic method. Teflon/PVA polymer will be first covalently functionalized with succinic acid via esterification of hydroxyl groups of PVA with the carboxylic groups of succinic acid. The succinic acid functionalized Teflon/PVA (f-SA-Teflon/PVA) will then be used to prepare the corresponding anthracene-SA-Teflon/PVA conjugate (Figure 36).

Figure 36. Chemistry of attachment of 9-hydroxymethyl anthracene to the Teflon/PVA surface.
Four different types of Teflon/PVA heterogeneous surfaces are proposed for synthesis using two different synthetic approaches. Compounds will be attached either to the plain or functionalized Teflon/PVA surface. The compounds to be attached are (1) 9-bromomethylandanthracene, (2) chlorin e6 (3) pheophorbide-spacer-alkene, and (4) 9-antracenemethanol. The Teflon-pheophorbide heterogeneous surface will be tested for drug photorelease and the amount of sensitizer release will be quantified.

4.2 Results

4.2.1 Alkylation of Teflon/PVA Surface Using 9-Bromomethylandanthracene. To establish optimal conditions for the covalent functionalization of Teflon/PVA surfaces, 9-bromomethylandanthracene was used as a model compound to first functionalize the Teflon-like surface. Figure 37 shows the synthetic scheme for 9-bromomethylandanthracene covalent attachment to the Teflon/PVA surface in the presence of NaH. In this reaction, hydride ions deprotonate the surface hydroxyl groups of PVA, rendering them more nucleophillic. Nucleophillic substitution of this deprotonate hydroxyl with an alkyl bromide completes the attachment.

![Synthetic Scheme](image)

**Figure 37.** Covalent attachment of 9-bromomethylandanthracene to the Teflon-PVA polymer has been shown where fluorine and hydroxyl groups are randomly distributed on the polymer surface.

First we synthesized 9-bromomethylandanthracene from 9-antracenemethanol using a similar synthetic method described previously. A suspension of 9-hydroxymethylandanthracene (1.5 g, 7.2 mmol, 1 equiv) in toluene (40 mL) was cooled to 0 °C, followed by addition of phosphorus...
tribromide (0.8 mL, 8.4 mmol, 1.17 equiv) via syringe. The mixture was stirred at 0 °C for 1 h and then warmed to room temperature, during which the reaction became homogeneous. Saturated Na₂CO₃ solution (15 ml) was added slowly and the reaction was stirred until it cooled to room temperature. The phases were separated, and the organic phase was washed with H₂O (10 mL), brine (10 mL) and dried over MgSO₄. The yellow filtrate was concentrated to minimum volume, then stored at 0°C for crystallization. The yellow needle-like solid was collected and dried in vacuum (1.24 g). The yellow 9-anthracenemethanol product was obtained with 91% yield. TLC was compared with the starting material and LC-MS was taken to confirm the molecular weight of the 9-bromo methyl anthracene.

**Figure 38.** Synthesis of 9-bromomethylantracene from 9 anthracene methanol in presence of PBr₃.

Our next step was to determine whether we can covalently immobilize 9-bromomethyl anthracene to the Teflon/PVA surface. Two different type of Teflon/PVA surfaces (6:1 or 4:1 Teflon/PVA) were used for synthetic covalent attachment. We speculate that a higher amount of 9-bromomethylanthracene will be loaded to the Teflon/PVA 4:1 surface vs Teflon/PVA 6:1 surface due to higher amount of PVA present on the Teflon/PVA 4:1 surface. To prepare the heterogenous surface, 9-bromomethylanthracene was added to 10mL toluene containing four Teflon/PVA pieces (0.28g each) and heated at 80 °C for 24 h. We observed that after reaction Teflon/PVA surface turned yellow. Polymer pieces were washed with toluene, DMF, ether and methanol to remove any 9-bromomethylanthracene from the surface, followed by Soxhlet extraction in THF. The modified Teflon/PVA probe tips maintained their color after solvent wash and Soxhlet extraction confirming the covalent attachment of the methyl anthracene to the surface, see Figure 39.
**Figure 39.** Teflon/PVA surface before (white) and after (yellow) 9-bromomethylandanthracene covalent attachment.

The waste solvents from each washing step were monitored for the presence of unreacted 9-bromomethylandanthracene. Only the first two washes contained unreacted material by visual assessment. No colored waste solutions were observed during the later steps or the Soxhlet extraction.

The amount of 9-bromomethylandanthracene loaded onto both surfaces was quantified by UV-Vis spectroscopy. We took advantage of Teflon/PVA instability in DMSO and completely dissolve the functionalized Teflon/PVA polymer in DMSO within 48 h. Solution turned yellow and UV-of the solution was taken. The amounts of 9-bromomethylandanthracene loaded onto the Teflon/PVA surfaces are summarized in Table 6.

**Table 22.** Moles of 9-bromomethylandanthracene attached to the Teflon-like surface.

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Moles of Bromoanthracene/ g polymer</th>
<th>Moles of Bromoanthracene attached/ g polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teflon/PVA 6:1</td>
<td>6.79x10^-4</td>
<td>3.76x10^-6 (3.76 µmole)</td>
</tr>
<tr>
<td>Teflon/PVA 4:1</td>
<td>2.75x10^-3</td>
<td>11.0 x10^-6 (11 µmole)</td>
</tr>
</tbody>
</table>

The amounts of 9-methylandanthracene loaded to the two types of Teflon/PVA surfaces were 3.76 µmole per gram of Teflon/PVA 6:1 and 11 µmole per gram of Teflon/PVA 4:1. Successful functionalization was observed using this strategy, and as expected the degree of attachment was higher for Teflon/PVA 4:1 surface which contained higher amounts of PVA on the surface.
4.2.2 Immobilization of Chlorin e₆ to the Teflon/PVA Surface via Nonphotocleavable Linker. A heterogeneous sensitizer-Teflon/PVA surface was synthesized by covalently attaching a PDT drug to the Teflon-like surface. Chlorin e₆ was chosen as our sensitizer due to its high cytotoxicity⁸ and wide use in PDT treatments. The covalent attachment to the surface was done via a non photocleavable linker, bromopropanol, due to little availability of the spacer alkene.

The two synthetic steps that were used to covalently attach chlorin e₆ to the Teflon/PVA surface are summarized in Figure 40 below. In the first step we covalently attached the sensitizer, chlorin e₆, to the bromopropanol linker via an esterification reaction by EDC and DMAP coupling; in the second step the linker-chlorin e₆-monoester was attached to the Teflon/PVA surface in presence of NaH.

![Diagram](image)

**Figure 40.** Proposed scheme for covalent attachment of chlorin e₆ to the Teflon/PVA surface via bromopropanol linker.

First synthetic step, reaction of chlorin e₆ with 1-bromo propanol, was achieved by reacting 10 mg chlorin e₆ (0.017 mmoles) with EDC (3.2 mg, 0.017 mmoles) and DMAP (2.36 mg, 0.017 mmoles) in CH₂Cl₂ (5mL), under stirring and N₂ atmosphere for 24h at room temperature.
Reaction was followed by HPLC analysis. Chromatogram of starting material, chlorin e₆, \( (t_R=12.6\, \text{min}) \) is shown in Figure 41, while the chromatogram of the final reaction mixture its shown in Figure 42. The profile chromatogram of the final reaction contains two peaks at 15.2 min and 15.6 min which reveal two products formation: chlorin e₆-monoester and chlorin e₆-diester.

Separation of the two reaction products, chlorin e₆ monoester and diester, was achieved by column chromatography. The chlorin e₆ monoester collected after column separation was analyzed by LCMS (see fig 38). The mass spectra revealed an intense peak for the expected chlorin e₆ monoester compound MS (+ESI) m/z calculated for \( \text{C}_{37}\text{H}_{41}\text{BrN}_4\text{O}_6 \left[\text{M+H}\right]^+ \) 712.5, found: 712.5.
Figure 41. HPLC chromatogram of the starting material, chlorin e₆, $t_R=12.6$ min.
Figure 42. HPLC chromatogram of the final reaction mixture after covalent attachment of bromopropanol linker to chlorin e₆ showing the formation of mono and diester.
Figure 43. Mass spectra of chlorin e₆ monoester.
Chlorin e₆-modified Teflon/PVA surface was prepared by using chlorin e₆ monoester for surface immobilization. In a typical reaction to the solid chlorin e₆ monoester, NaH (0.015 mmol) 30 ml of dry toluene and three pieces (average weight 0.32 g) of Teflon/PVA were added to 50 ml round bottom flask. Reaction mixture was refluxed for 24 h under nitrogen atmosphere and Teflon/PVA surface turned green color. After reaction the newly prepared surface was washed with several solvents (DCM, THF, MeOH, toluene, hexane) and Soxhlet extracted using boiling methanol for 24 h to remove any physically adsorbed sensitizer. No sensitizer leaching was observed from the solids after Soxhlet extraction in methanol. The Teflon/PVA surfaces maintained their green color characteristic to chlorin e₆. We concluded that the sensitizer was covalently attached to the Teflon/PVA surface.

Figure 44. Teflon/PVA surface after chlorin e₆ covalent attachment.

The amount of covalently attached sensitizer to the Teflon/PVA surface was determined by dissolving the hybrid Teflon/PVA surface in DMSO. Polymer pieces were immersed in 1 mL of DMSO for 48 h, in which time the polymer completely disintegrated. DMSO solution turned green and UV of the solution was taken. The amount of sensitizer anchored to the surface was calculated from the calibration plot (calibration plot of absorbance of Soret band and known concentration of sensitizer in DMSO was generated). The amount of loaded sensitizer calculated using the above mentioned procedure was 46 nmol per 0.32 g cap or 144 nmol/g of Teflon/PVA.

4.2.3. Covalent Attachment of Pheophorbide to the Teflon/PVA Polymer. The work reported here describes efforts in covalent attachment of a PDT drug, pheophorbide-a (PPa), to the
newly synthesized Teflon/PVA surface via a photocleavable linker (spacer alkene). The efficiency of sensitizer to cleave free from the Teflon/PVA surface was also determined.

Pheophorbide-$a$ was chosen as the anchoring photosensitizer since it exhibits a Q-band absorption maximum at 660 nm, which makes it ideal for use in the tissue environment. It is known from literature that light within 600-700 nm region penetrates 50-200 % more (higher optical penetration depth) than light within 400-500 nm region. In PDT, pheophorbide-$a$ is known to be localized in mitochondria and induce cell death by apoptosis or necrosis. We have used Z spacer alkene as a substrate for singlet oxygen, as rate of reaction of singlet oxygen with the 16 electron rich spacer alkene is higher than with the E-alkene. For this work the Z-alkene was synthesized by my colleague, Dr. Goutam Gosh.

The synthetic steps used for immobilization of the sensitizer/spacer alkene to the Teflon-like surface are shown is Figure 45 below. First the hydroxyl group of the PPa monoester was converted to $\text{Br}$ by treatment with $\text{PBr}_3$ in THF and then the Br-PPa-monoester was attached to the Teflon-like surface in presence of NaH and THF, with 24h reflux, using a similar experimental procedure published previously by our group.

**Figure 45.** Synthetic approach for Teflon/PVA surface functionalization with PPa sensitizer.
Reaction completion of Br-spacer alkene/sensitizer synthesis was monitored by TLC and also verified by the presence of the product peak Br-PPa-monoester \((t_R = 26.6 \text{ min})\) and the absence of starting material, OH-PPa-monoester, \((t_R = 20.97 \text{ min})\) in the HPLC chromatogram of the crude reaction mixture.

Covalent bonding of the sensitizer/spacer alkene to the Teflon/PVA was accomplished by adding Br-PPa-monoester (8 mg, 0.0102 mmol) and NaH (0.245 mg, 0.0102 mmol) in 5 mL of dry THF containing our Teflon/PVA surface and refluxing the mixture at 70 °C for 24 h. The white colored probe tips turned brown-black (see Figure 40) after pheophorbide was covalently attached to the glass.

![Image of Teflon/PVA surface before (white) and after (dark brown) sensitizer attachment.](image)

**Figure 46.** Teflon/PVA surface before (white) and after (dark brown) sensitizer attachment.

The hybrid probe tips were subjected to the solvent wash and Soxhlet extraction. Solid was stable in the dark, no sensitizer leaching was observed when the material was repeatedly washed with toluene, THF, chloroform, ether, and hexane solvents and Soxhlet extracted with chloroform and ethanol. Because we didn’t detect any sensitizer in the filtrates we concluded that sensitizer was covalently attached onto the Teflon/PVA surface.

Sensitizer loading: The amount of Pheophorbide attached onto the Teflon/PVA surface was determined by UV. The heterogeneous surface was placed in 1 ml of DMSO for 48h, time in which the functionalized Teflon/PVA surface completely disintegrated, turning the clear DMSO solution to a light brown color, which is characteristic to pheophorbide. The UV of the solution was taken and the amount of PPa loaded to the Teflon/PVA surface was determined to be 134 nmoles per 0.447 g probe tip.
A key step in our PDT method is sensitizer departure from the Teflon/PVA surface. The amount of sensitizer photorelease from the surface in solution was quantified. The photocleavage study was conducted by placing 0.447 g of Teflon/PVA, previously loaded with 134 nmoles of sensitizer, in 1mL octanol. Oxygen was bubbled for 20 min in the solution and samples were irradiated at 669 nm for 2 h. With 134 nmoles attached to the Teflon/PVA surface we observed (see Table 23 and Figure 47) that photorelease in octanol reached ~99% in 100 minutes.

**Table 23.** Nanomoles and percent sensitizer release from the Teflon/PVA surface in 1.0 mL butanol after 2 h. Amount of sensitizer present in solution was detected by UV.

<table>
<thead>
<tr>
<th>time</th>
<th>nmol released</th>
<th>% released</th>
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<tbody>
<tr>
<td>10</td>
<td>27</td>
<td>20.1</td>
</tr>
<tr>
<td>20</td>
<td>36</td>
<td>26.9</td>
</tr>
<tr>
<td>30</td>
<td>75</td>
<td>56.0</td>
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<tr>
<td>40</td>
<td>88</td>
<td>65.7</td>
</tr>
<tr>
<td>50</td>
<td>102</td>
<td>76.1</td>
</tr>
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<td>60</td>
<td>103</td>
<td>76.9</td>
</tr>
<tr>
<td>70</td>
<td>115</td>
<td>85.8</td>
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<tr>
<td>80</td>
<td>132</td>
<td>98.5</td>
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<tr>
<td>90</td>
<td>132</td>
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<td>100</td>
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<td>110</td>
<td>133</td>
<td>99.3</td>
</tr>
<tr>
<td>120</td>
<td>133</td>
<td>99.3</td>
</tr>
</tbody>
</table>

Figure 47 shows the amount (nmoles) of sensitizer photoreleased into octanol solution, as a plot of sensitizer release versus time. We observed that photooxidation and dioxetane cleavage were fast in octanol. In 100 minutes most of the pheophorbide sensitizer (133 nmoles) departed from the Teflon/PVA surface when surface was loaded with 134 nmoles of sensitizer.
Figure 47. Time profile for the photocleavage of 133 nmol sensitizer from the Teflon/PVA surface into 1.0 mL of 1-octanol

Our previous adsorption studies can explain the high percent of drug photocleaved from the Teflon/PVA surface. When surface ability to resist drug adsorption was evaluated we noticed that its resistance was very high. We observe that when the Teflon-like surface was immersed in PPa containing solution for 3 h only 3.8 nmoles (1.95 %) from the PPa present in solution adsorbed to the Teflon/PVA surface. Such high yield of sensitizer release from the surface is due to Teflon/PVA resistance to drug adsorption, increasing the amount of sensitizer released in solution. We successfully observed that the Teflon-like surface can serve efficiently as sensitizer support and has the ability to release the pheophorbide drug from its surface.

4.2.4 Teflon/PVA Surface Modification: Functionalization of Teflon/PVA with Succinic acid. Succinic acid (SA) was covalently attached to the Teflon/PVA surface by an esterification reaction (Figure 48) following experimental conditions of Salavagion et al.11 with some modification. The hydroxyl groups of PVA are esterified with the carboxylic groups of succinic acid via EDC- DMAP coupling.
In a typical reaction, the succinic $0.100 \text{ g (0.0085 moles)}$ and $0.422 \text{ g Teflon/PVA}$ were placed in $15\text{ml anhydrous dichloromethane}$ and $0.081 \text{ g (0.000425 moles)}$ EDC and $0.052 \text{ g (0.000425 moles)}$ DMAP were added. The reaction was stirred under nitrogen atmosphere for 5 days. Samples were washed with different solvents and Soxhlet extracted in methanol for 24h.

The IR spectra of plain Teflon/PVA (Figure 49), succinic acid functionalized Teflon/PVA (Figure 50) and Succinic acid (Figure 51) were collected and compared to confirm the covalent attachment of succinic acid to the Teflon/PVA surface.

**Figure 48.** Functionalization of Teflon/PVA with succinic acid by esterification reaction

**Figure 49.** The FTIR spectrum of plain Teflon/PVA in which the 3100-3600 cm$^{-1}$ band is assigned to O-H stretching (characteristic to PVA) while the bands near 1203 and 1147 cm$^{-1}$ were assigned to the C-F stretching vibrations of PTFE.

Measurements of Teflon/PVA material were carried out using an FTIR spectrometer (Mattson, 6020-Galaxy Series) in transmittance mode in the frequency range of 4000–700 cm$^{-1}$. 
The IR spectra of the unmodified Teflon/PVA shows two bands near 1203 and 1147 cm\(^{-1}\) which were assigned to the C-F stretching vibrations of PTFE while the two bands around 2937 and 3315 cm\(^{-1}\) are characteristic for PVA. The broad band observed from 3100-3600 cm\(^{-1}\) may be assigned to O-H stretching. The 2850-2950 cm\(^{-1}\) band is assigned to C-H alkyl stretching. The peaks at 1419-1325 cm\(^{-1}\) are attributed to stretching vibration of C-O of the acetate group, remained unhydrolized in the PVA polymer.

Figure 50. Infrared spectrum of Teflon/PVA esterified with succinic acid showing the ester absorption at 1717 cm\(^{-1}\).
Figure 51. Infrared spectrum of succinic acid.

For the succinic acid IR spectra (Figure 51) the 3300-2500 cm⁻¹ broad stretching vibration was assigned to O–H group, the 2635 and 2534 cm⁻¹ were assigned to C–H stretching, while the 1692 cm⁻¹ to C=O group.

FTIR analysis of the modified Teflon/PVA shows evidence of covalent attachment of succinic acid onto the surface (See Figure 44). The absorption at 1717 cm⁻¹ corresponds to the stretching vibration of C=O ester group formed between the alcohol groups of PVA and carboxylic acid groups of succinic acid which is absent in the unmodified Teflon/PVA spectrum. The band appears weak in the esterified polymer due to the low content of succinic acid with respect to the polymer.

The amount of succinic acid attached was determined by NMR. Benzene is used as an internal standard and peak integration of succinic acid with respect to benzene was used to calculate the amount of succinic acid present before and after reaction. The calculated amount of succinic acid attached was 18.2 µmoles per 0.422 g polymer or 43.12 µmoles per gram Teflon/PVA. (See Table 20).
Table 24. Loading of succinic acid onto the Teflon/PVA surface.

<table>
<thead>
<tr>
<th>Amount of Teflon/PVA (g)</th>
<th>Amount succinic acid attached (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.422</td>
<td>18.2</td>
</tr>
<tr>
<td>1.0</td>
<td>43.12</td>
</tr>
</tbody>
</table>

4.2.4.1 Covalent Attachment and Loading of 9-Antracenemethanol to the Succinic Acid Functionalized Teflon/PVA Microtip. Hybrid Teflon/PVA probe tip was synthesized by covalently attaching a model compound, 9-antracenemethanol, via an esterification reaction to the Teflon/PVA surface, which was previously modified with succinic acid. Synthetic scheme used for surface immobilization of 9-antracenemethanol is shown in Figure 52. Covalent attached of 9-antracenemethanol to the succinic acid functionalized Teflon/PVA was achieved via an esterification reaction. Hydroxyl groups of 9-antracenemethanol were esterified with the carboxylic groups of succinic acid via EDC, DMAP coupling.

![Chemical structure](image.png)

Figure 52. Attachment of 9-antracenemethanol to the functionalized Teflon/PVA surface.

In a typical reaction the functionalized Teflon/PVA polymer was placed in a 15mL dichloromethane with 0.053 g of 9-antracenemethanol (2.5x10^{-4} moles), 0.023 g EDC (0.00012 moles) and 0.015 g (0.00012 moles) DMAP. The reaction mixture was stirred under nitrogen at room temperature for 5 days.

After reaction polymer pieces were washed with dichloromethane, methanol and then Soxhlet extracted for 12 h in methanol. The polymer surface turned yellow and maintained its color after solvent wash and Soxhlet extraction (Figure 47). During Soxhlet extraction the hot boiling solvent
removes any adsorbed 9-anthracenemethanol from the surface. After the wash we observed that the heterogeneous surface still maintained its yellow color; hence, we concluded that 9-antracenemethanol was covalently attached to the Teflon/PVA surface.

![Figure 53. Succinic acid functionalized Teflon/PVA surface, before (white) and after (yellow) 9-antracenemethanol covalent attachment.](image)

Figure 53. Succinic acid functionalized Teflon/PVA surface, before (white) and after (yellow) 9-antracenemethanol covalent attachment.

![Figure 54. UV spectrum of DMSO solution containing the dissolved anthracene functionalized Teflon/PVA surface, absorbance maxima 366 nm.](image)

Figure 54. UV spectrum of DMSO solution containing the dissolved anthracene functionalized Teflon/PVA surface, absorbance maxima 366 nm.

To determine the amount of anthracene loaded to the heterogeneous polymer, the surface was immersed in 0.7 ml of DMSO solution for 48 h. The anthracene-succinic-acid-Teflon/PVA surface completely disintegrated in DMSO after 48 h and solution turned pale yellow. The UV of the
DMSO solution was taken, and the absorption was followed at 366nm (Figure 50). The amount of 9-antracenemethanol attached to the surface was determined from a calibration curve which was prepared from five standard solutions of different concentration (4.8×10⁻⁵ M, 6.0×10⁻⁵ M, 7.3×10⁻⁵ M, 8.4×10⁻⁵ M and 9.6×10⁻⁵ M). We determined that the amount of 9-antracenemethanol loaded to 0.422 g Teflon/PVA surface was 8.15 µmoles or 19.31 µmoles per 1 g of Teflon/PVA.

![Calibration plot of 9-antracenemethanol in DMSO.](image)

4.3 Conclusion. Four different compounds were successfully covalently attached to the new Teflon/PVA surface via a photoliable or non-photoliable linker using either plain Teflon/PVA or succinic acid modified Teflon/PVE surface. We successfully synthesized (1) 9-bromomethyl anthracene Teflon/PVA modified surfaces via an alkylation reaction and 3.76 µmoles of 9-bromomethyl anthracene were anchored per gram of Teflon/PVA (2) a heterogeneous sensitizer-Teflon/PVA surface, whereas 144 nmol of chlorin e₆ was directly anchored to one gram of plain Teflon/PVA surface via a non-photocleavable bromopropanol linker. (3) a hybrid Teflon/PVA-9-antracenemethanol probe tip by covalently attaching 9.31 µmoles of 9-antracenemethanol via an esterification reaction onto Teflon/PVA surface, which was previously modified with succinic acid and (4) a sensitizer-Teflon/PVA surface containing a covalently attached pheophorbide molecules which was immobilized to the surface via a photodetachable linker. The heterogeneous surface when placed in solution, in
presence of light and oxygen, was observed to show coloration of butanol solution where ~ 99 % sensitzer detached from the probe tip.

The results reported here provide knowledge of the strategies that can be applied for covalent attachment of model compounds or sensitizers to a Teflon-like surface, and the ability of the sensitzer to be photoreleased from the surface in presence of light and oxygen. The surfaces can be used as sensitzer support and have the option to be coupled with the microoptic PDT device which is used for human cancer cell killing.

4.4 Reference
Chapter 6. Photooxidation reactions at silica surface

6.1 Introduction. Photochemical oxidation reactions which are known to be toxic to organisms and destructive to materials, can be efficiently used for water disinfection. Because the photosensitized oxidation produces a number of intermediates, (see fig. 56) which are generated in different amounts over time, it is challenging to study the oxidative process. Deducing the contribution of each intermediate for water disinfection remains a challenge. Also, species formed in photooxidation reactions are very unstable and their quantification is challenging.

Figure 56. Intermediates generated in the photosensitized oxidation process.

Figure 56 also shows an initial \(^{1}\text{O}_2\) reaction where RO–OR builds up in concentration and can be sensitized to decompose to RO\(^{\cdot}\). Chemists have had no way to get at such important mechanistic detail, so our aim is to start working towards those findings. We propose for development to use bi- and triphasic systems with regions that are controllably dry, partly wetted, and/or fully wetted (see Figure 57). Our technique physically isolates sensitizer and peroxide molecules at an interface; this allows to accurately quantitate \(^{1}\text{O}_2\) and RO\(^{\cdot}\) transport from a fluorinated silica or polydimethylsiloxane (PDMS) superhydrophobic sensitizer surface into solution.

We aim to anchor sensitizer and peroxide molecules to silica surfaces in order to enable them to selectively produce \(^{1}\text{O}_2\) or RO\(^{\cdot}\); clean and pure. The very nature of \(^{1}\text{O}_2\) chemistry is to add to alkenes to initially generate O–O bonds; but a second triplet-triplet energy transfer from sensitizer to the O–O bond to homolyze it is almost always neglected. This second energy transfer step can occur when the sensitizer triplet energy is above the peroxide O–O bond dissociation energy, and when properly designed in a material, it can be exploited. The working hypothesis is that when the triplet sensitizer energy is > 5 kcal/mol above the peroxide O–O bond dissociation energy, it will initiate
RO$^\cdot$ release from the silica surface, and that $^1$O$_2$ physical quenching will be reduced with surface fluorination. This will allow us to study reactive oxygen in isolation.

![Image](image.png)

**Figure 57.** Image of the particles modified to release singlet oxygen and alkoxy radicals upon visible light irradiation. The particles are in contact with solution (biphasic) or in a superhydrophobic surface (triphasic). Singlet oxygen and alkoxy radicals are produced in the vicinity of the sensitizer, which are fully wetted or partially wetted or dry before departing into the solution.

Until present only relatively few studies$^{1-5}$ have focused on photosensitized dissociation of peroxides. Energy transfer from sensitizer is likely to require thermal activation for O–O bond dissociation,$^{6-12}$ which has been observed by Scaiano et al.$^5$ for energy transfer to repulsive excited states of di-tert-butylperoxide.

We will first start our study using non-covalently attached sensitizer and peroxide particles to confirm the photosensitized dissociation of peroxide by triplet sensitizer energy transfer. We propose to (1) prepare adsorbed sensitizer and peroxide silica particles with different loadings (2) initiate photosensitized dissociation of peroxides, upon sensitizer irradiation, and (3) desorb the adsorb particles in solution (4) and quantify the amount of peroxide dissociated.

6.2 Results and discussion

6.2.1 Synthesis of Sensitizer and Peroxide Silica Adsorbed Particles. Our first goal was to determine whether photosensitized dissociation of peroxides will take place at the fumed silica
surface by triplet sensitizer energy transfer. We first started our study using non-covalently attached (adsorbed) sensitizer and peroxide particles to the fumed silica surface. Six types of surfaces were prepared containing adsorbed sensitizer and peroxide with different loading: Dicumyl Peroxide to 4,4’-Dimethyl benzyl 1:1, 10:1, 40:1, 100:1, 500:1 and 1000:1. The last three surfaces, peroxide:benzyl 100:1, 500:1 and 1000:1 were synthesized by my colleague Niluksha Walalaela.

The amounts of adsorbed sensitizer and peroxide on fumed silica particles with different loadings are summarized in Table 25. Surface 1 (Peroxide : benzyl 1:1) was synthesized by first preparing a solution containing 23.8 mg (0.1 mmol) 4,4’-dimethyl benzil and 27 mg (0.1 mmol) dicumyl peroxide in 15 ml dichloromethane and then 300 mg fumed silica was added to it. Sensitizer and Peroxide were allowed to absorb to the fumed silica surface for 1 h in the dark and then the solution was filtered out and silica was dried under vacuum. Surface 2 (Peroxide : benzyl 10:1) and surface 3 (Peroxide : benzyl 40:1): were prepared by adding 27 mg (0.1 mmol) dicumyl peroxide for each surface to 2.38 mg (0.01 mmol) 4,4’-dimethyl benzil for surface 2 or 0.595 mg (0.0025 mmol) of 4,4’-dimethyl benzil for surface 3 in 15 ml dichloromethane. To the sensitizer and peroxide solutions, 300 mg of fumed silica was added for each and kept for 1 h in the dark. Solution was filtered out and silica was dried under vacuum.

Table 25. Amounts of dicumyl peroxide and 4,4’-dimethyl benzyl adsorbed on fumed silica particles.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Peroxide : benzyl</th>
<th>mmole/ 300g SiO2</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface 1</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.1</td>
<td>1:1</td>
</tr>
<tr>
<td>Surface 2</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.01</td>
<td>10:1</td>
</tr>
<tr>
<td>Surface 3</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.0025</td>
<td>40:1</td>
</tr>
<tr>
<td>Surface 4</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.001</td>
<td>100:1</td>
</tr>
<tr>
<td>Surface 5</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.0002</td>
<td>500:1</td>
</tr>
<tr>
<td>Surface 6</td>
<td>Peroxide : benzyl</td>
<td>0.1 : 0.0001</td>
<td>1000:1</td>
</tr>
<tr>
<td>Control surface</td>
<td>Peroxide only</td>
<td>0.1</td>
<td>-</td>
</tr>
</tbody>
</table>
Control silica surface containing 0.1 mmole peroxide only was also prepared by adding 27 mg (0.1 mmole) of Dicumyl Peroxide to 300 mg of fumed silica particles in 15mL of dichloromethane. Peroxide was allowed to absorb to the fumed silica surface for 1 h in the dark and then the solution was filtered out. After filtration the adsorbed silica particles were dried under vacuum.

6.2.2 Photosensitized Dissociation of Peroxide. For the photochemical reaction, fumed silica particles containing adsorbed dicumyl peroxide and 4,4’-dimethyl benzyl in different ratios were used to test the photosensitized dissociation of peroxide. In a typical reaction, 100 mg of sensitizer and peroxide adsorbed silica particle were placed in a vial and capped. Vial was irradiated for 1 h using two metal halide lamps and allowed to rotate horizontally using mechanical stirrer, so that particles can mix with each other by tumbling motion. Control reactions were also prepared where the same amount of particles (100 mg) were placed in vials and allowed to rotate horizontally using mechanical stirrer, without irradiation in the dark.

After photolysis the adsorbed peroxide and sensitizer particles were desorbed in solution. 1.4 ml of acetonitrile was added to the control and the irradiated sensitizer-peroxide-silica particles, and allowed to sit for 1 h in the dark. The adsorbed peroxide and sensitizer desorbed back in the acetonitrile solution, which was filtered and analyzed by GC-MS.

Upon GC-MS analysis of the desorbed solutions of the sensitizer and peroxide SiO₂ before and after irradiation, we successfully observed photosensitized dissociation of dicumyl peroxide. In the GC-MS spectra of photolyzed samples (fig. 59), we found a new mass of a fragment 119.1 (~16min) which showed a new product formation from peroxide dissociation. The new peak was absent when particles were not irradiated (fig. 58). The findings in the GSMS chromatograms confirmed that we were able to successfully initiate photosensitized dissociation of peroxides, upon sensitizer irradiation.

Our hypothesis was that when the triplet sensitizer energy is >5 kcal/mol above the peroxide O–O bond dissociation energy an RO• release will be initiated from the silica surface. The triplet sensitizer energy of the 4,4’-dimethyl benzyl (51 kcal/mol) being 17 kcal/mol above the dicumyl peroxide O–O bond dissociation energy (34 kcal/mol), allowed for initiation of RO• release from the silica surface which we were able to detected by GCMS.
Figure 58. CG-MS spectra of desorbed peroxide and sensitizer SiO$_2$ particles before photoirradiation in acetonitrile.
Figure 59. CG-MS spectra of desorbed peroxide and sensitizer SiO$_2$ particles after photoirradiation in acetonitrile-d sowing new peak appearance at ~ 16min, confirming the photosensitized dissociation of peroxides.
6.2.3 Peroxide Quantification after Particle Desorption in Acetonitrile. To quantify the amount of peroxide dissociated, the percent conversion was calculated from the GCMS chromatogram based on peroxide peak disappearance. Peak area of dicumyl peroxide was compared with peak area of biphenyl, which was used as an internal standard. For analysis 185 μL of desorbed sample was mixed with 15 μL of internal standard (Biphenyl-MW154).

We observed that the amount of photosensitized dissociated peroxide varies with the SiO$_2$ particle loading. As seen from Table 26, the percent conversion ranged from 1.18 % to 25.3 %; so there seems to be an optimal ratio of sensitizer and peroxide loading for maximum peroxide dissociation.

Table 26. Percent conversion calculated based on peroxide peak disappearance (average of 3 experiments). Biphenyl was used as internal standard.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Peroxide : benzyl</th>
<th>ratio</th>
<th>Initial</th>
<th>Final</th>
<th>Peak decrease</th>
<th>% conversion</th>
<th>% enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peroxide : benzyl</td>
<td>1:1</td>
<td>1.38±0.07</td>
<td>1.26±0.05</td>
<td>0.120</td>
<td>8.70</td>
<td>5.28</td>
</tr>
<tr>
<td>2</td>
<td>Peroxide : benzyl</td>
<td>10:1</td>
<td>1.35±0.03</td>
<td>1.01±0.01</td>
<td>0.342</td>
<td>25.3</td>
<td>21.9</td>
</tr>
<tr>
<td>3</td>
<td>Peroxide : benzyl</td>
<td>40:1</td>
<td>1.64±0.05</td>
<td>1.51±0.01</td>
<td>0.135</td>
<td>8.19</td>
<td>4.77</td>
</tr>
<tr>
<td>4</td>
<td>Peroxide : benzyl</td>
<td>100:1</td>
<td>1.93±0.04</td>
<td>1.83±0.09</td>
<td>0.104</td>
<td>5.40</td>
<td>1.98</td>
</tr>
<tr>
<td>5</td>
<td>Peroxide : benzyl</td>
<td>500:1</td>
<td>2.15±0.03</td>
<td>2.13±0.03</td>
<td>0.025</td>
<td>1.18</td>
<td>-2.24</td>
</tr>
<tr>
<td>6</td>
<td>Peroxide : benzyl</td>
<td>1000:1</td>
<td>2.18±0.27</td>
<td>2.08±0.04</td>
<td>0.099</td>
<td>4.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Control</td>
<td>Peroxide only</td>
<td></td>
<td>1.34±0.08</td>
<td>1.29±0.02</td>
<td>0.458</td>
<td>3.42</td>
<td></td>
</tr>
</tbody>
</table>

The highest amount of dissociated peroxide was achieved when silica particles were loaded with dicumyl peroxide and 4,4’-dimethyl benzyl in 10:1 ratio. We speculate that a higher or lower ratio of sensitizer to peroxide loading reduces the peroxide dissociation efficiency, and was attributed to less available sensitizer and self-quenching, respectively.

6.3 Conclusion. Triplet sensitizer energy transfer as a strategy to break peroxide O–O bonds for RO$^-$ release was confirmed. We successfully observed the photosensitized dissociation of
dicumyl peroxide upon irradiation of 4,4'-dimethyl benzyl sensitizer adsorbed on fume silica particles. Particles were prepared by adsorbing sensitizer and peroxide onto silica surface in different ratios: dicumyl peroxide:4,4’-dimethyl benzyl 1:1, 10:1, 40:1, 100:1 500:1 1000:1. After particle irradiation and desorption, GCMS of the reaction mixture was analyzed. The results obtained for the photosensitizer and peroxide adsorbed silica particles without irradiation, were compared with the photosensitizer and peroxide adsorbed silica particles with irradiation. The highest amount of cleaved peroxide (25.3%) was detected in solution when particles were loaded with dicumyl peroxide: 4,4’-dimethyl benzyl in 1:10 ratio.

Our next step will be to look into photochemical and surface science reactions confining the sensitizer and peroxide molecules on surfaces. We will test photoreactions of $^1$O$_2$ where alkoxy radicals (RO∙) also come into existence via sensitized homolysis of peroxide sites on the surface. All reactions will be photosensitized; the reagents and products will not be directly irradiated. The bi- and triphasic sensitizers will produce key insight and a more rigorous approach to defining photooxidation mechanisms and relative participation of reactive oxygen. The development of a heterogeneous system to generate singlet oxygen and alkoxy radicals, and then separate them from otherwise complex mixtures would be mechanistically useful.

6.4 Reference
8. Ng, N. C.; Guillet, J. E. Macromolecules 1978, 11, 937942